The natural decline of potato cyst nematodes in the absence of a host crop and their movement by cultivation operations

Thesis

The natural decline of potato cyst nematodes in the absence of a host crop and their movement by cultivation operations

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ABSTRACT

Decline of potato cyst nematode populations in the absence of a host crop and movement by cultivation operations

This thesis has investigated the variation in PCN population density declines in the absence of a host crop and, the movement of PCN by cultivation operations in a potato rotation.

The use of crop rotation as a management strategy for the control of potato cyst nematodes (PCN) is dependent on the natural decline rate of PCN. Any substantial variation among PCN populations will influence the adequacy and duration of crop rotation. Two experiments were undertaken to assess the extent of variation in PCN decline. Experiment 1 consisted of the annual sampling of semi-permanent field test stations to monitor the population decline in five infested fields, over four years. Large variations within the PCN population densities were found over the duration of the experiment, these included apparent increases in population density. These increases were not found for the controls in the field boundaries. The observed variations were probably due to the net movement of cysts in and around the stations as a result of soil movement by cultivation, as identified in further studies. Experiment 2 consisted of 45 plunge pits with soil from different PCN infested fields. The plunge pits were sampled at four monthly intervals, for twenty months. The range of decline was 11 to 69 % per annum. The variation in decline rates between the populations could not be accounted for by differences between *G. pallida* and *G. rostochiensis* populations or completely by soil type. This suggested that decline information was required on a site-specific basis and that sampling within a field is not reliable if cultivations are carried out between sampling dates.

Further studies determined that PCN cysts are moved by cultivation operations. With the exception of the bed-former, all cultivations investigated were found to result in the movement of PCN. The range of movement by a cultivation operation was from within 1 m up to 5 m. The cultivation operation employed and its purpose determine the direction and extent of cyst movement.

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AUTHOR'S DECLARATION

This thesis is the work of, and has been written by, the author. No part of this work has been submitted for any other degree or professional qualification.

Alasdair B. Haley

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1 Introduction to potato cyst nematodes

Potato cyst nematodes (PCN) belong to the genus *Globodera*, of the family Heteroderidae, which contains some of the most specialised and economically damaging nematodes in global agriculture. Two closely related species of potato cyst nematodes are currently recognised, *Globodera rostochiensis* and *G. pallida* (Turner and Evans, 1998). Potato cyst nematodes co-evolved with their host plant, the potato, in South America several hundred thousand years ago, and are highly specialised parasites, mainly attacking root systems (Stone, 1985).

Early work suggested that separation of the two species occurred less than 100,000 years ago (Stone, 1979). However, more recent molecular work suggests that species separation actually occurred millions of years ago (Bakker and Bouwman-Smits, 1988). Since speciation, a number of races and pathotypes with differing levels of virulence have developed within both species (Canto Saenz and de Scurrah, 1977).

1.1 Origins and coevolution of PCN

Contrasting views exist as to the location in South America where *G. rostochiensis* and *G. pallida* originated and distribution of both species is not uniform. Research carried out by Evans, Franco and de Scurrah (1975) found *G. rostochiensis* to be the dominant species in PCN populations in the southern latitudes of the Andes, with only *G. pallida* present in PCN populations north of latitude 15.6^{o} S. This work also suggests that the Peruvian/Bolivian Andes is a centre of origin for PCN. Northwest Argentina is another possible origin for PCN as it has the highest number of wild potato species exhibiting resistance to PCN (Franco, 1977; Turner, 1989). Due to the migration of the species throughout South America it is difficult to pinpoint the origin. After molecular analysis on PCN populations, Ferris *et al.* (1995) suggested that the origin of PCN was in Mexico. It is hard to determine which theory is correct but further investigations may provide more

solid evidence, such as looking at the resistance of wild potatoes in Mexico and molecular diagnostics of Argentinian PCN populations.

The evolution of the two species is also difficult to trace as they are today found in mixed populations competing for the same resources (Stone, 1979). However, for speciation to occur, some physical or physiological barrier must have been present to allow sufficient divergence in populations. Several hypotheses have been conceived to answer this, one suggestion being that physiological adaptation to changes in day length and temperature between populations resulted in speciation (Evans, Franco and de Scurrah, 1975). These differences in day length and temperature will be discussed later (Section 1.7.3). Due to the high altitude of the tropical regions where cysts probably originated they are naturally suited to the temperate climate of Europe. However, they have also adapted to and become serious pests of potato crops in some sub-tropical areas (Jones, 1961).

Stone (1979) suggests that speciation occurred when PCN spread north from Argentina and occupied both sides of Lake Titicaca, resulting in a physical barrier between populations, with only *G. pallida* populating the colder northern regions, and subsequently moving south to mix with the *G. rostochiensis* populations. Brücher (1959) and Franco (1977) hypothesised that speciation occurred during glaciation, due to populations being separated by the barrier of ice with only *G. pallida* to the north of Lake Titicaca.

1.2 Geographical distribution

The spread of PCN on a global scale is mainly due to the success of its host plant as a crop, with potatoes grown in over 70 countries (FAO, 2001). The exact date of introduction of PCN into Europe is not known, but it is thought to have occurred during the mid-nineteenth century, after expeditions were sent to South America to bring back cultivars with *Phytopthera infestans* (late potato blight) resistance.

The first recorded case of cyst-forming nematodes on potatoes was by Kühn in 1881, but

the nematode was identified as *Heterodera schachtii* (sugar-beet cyst nematode). By the early twentieth century the nematode had been recorded as damaging potato crops throughout Europe. In 1923, Wollenweber first separated the potato cyst nematode (round cysts) as a different species from the sugar-beet cyst nematode (lemon-shaped cysts), using morphological differences. The new species was named *Heterodera rostochiensis Woll.* after the area of Germany (Rostock) where it was first recognised.

By 1970, differences of morphology within PCN populations began to be noted, resulting in questions as to the homogeneity of *H. rostochiensis* (Guile, 1970; Evans and Webley, 1970; Stone, 1972a). At the simplest level, it was noticed that all females newly emerged from the potato root were white, but that the length of time they remained white before becoming a tanned colour differed. The earlier to changed colour, golden cyst nematodes, retained the species name *H. rostochiensis*; those that change colour later, the white cyst nematodes, were separated as a new species, *Heterodera pallida* (Stone, 1972b). Further changes in classification resulted, placing PCN and closely related species in a distinct genus from *Heterodera*, the new genus being named *Globodera* (Mulvey and Stone, 1976). The spread of PCN globally (except for one example) seems to have resulted initially by primary transport of PCN to Europe, followed by subsequent movement of contaminated material to other regions of the world. The only recorded example of direct contamination from South America to a non-European country is the movement of cysts from Chile to Japan in guano sacks (Inagaki and Kegasawa, 1973). Populations of PCN can now be found in 65 countries (EPPO, 1994).

In a recent survey by Minnis *et al.* (2002), it was found that approximately 64% of the land currently used for potato production in the UK is infested with PCN. Of the fields found to be infested, 92% contained *G. pallida*, with 67% containing only this species; 33% of the fields contained *G. rostochiensis*, but only 8% contained pure *G. rostochiensis* populations.

1.3 Identification of PCN

Separation of *Globodera* from *Heterodera* is possible using cyst morphology as PCN have round cysts with no vulval cone, whereas the *Heterodera* species are lemon shaped and usually have a prominent vulval cone. This visual difference may become less clear, depending on the age and damage to the cyst in the soil. Problems can be found in samples that also have *Punctodera* (grass cyst nematodes) cysts present, as they closely resemble the shape of *Globodera.* When both *Globodera* and *Punctodera* are present they can normally be differentiated due to the *Globodera* cysts being more spherical in shape. Additionally, examination of the anal region of the *Punctodera* will result in two distinct fenestrae being seen (Hesling, 1978).

The two species of PCN can be separated by the colour of newly emerged females but this is not a reliable method of species separation due to both species cyst turning a tan colour as they mature so, the cysts have a tendency to be different shades depending on soil type. These species can be separated in more detailed analysis of morphological differences in the juvenile life-cycle stages, such as differences in the stylet size (*G. pallida* more than 21.8 μm) and number of ridges between anus and vulval basin (*G. pallida* usually less than 14 μm) (Hesling, 1978).

PCN species can also be distinguished using isoelectric focusing (IEF). This technique utilises on the migration of two species-specific proteins along a pH gradient on a polyacrylamide gel, to specific isoelectric (pI) points. A protein of pI 5.7 is found in *G. pallida* and one of pI 5.9 in *G.rostochiensis* (Fleming and Marks, 1982). This technique has constraints if contaminants are present or only small amounts of testable sample are available. The identification of species-specific DNA sequences in the genome has allowed the use of polymerase chain reaction (PCR) as a technique for identifying the species present. This technique uses a specific primer to isolate a specific part of the genome

where differences between the two species have been identified, and then replicates the species-specific sequence (Sambrook, Fisch and Maniatis, 1989). This technique is being constantly improved using different chemicals and enzymes, and appears to predict PCN species composition in a population to an accuracy of 5%. Polymerase chain reaction techniques can be used on small amounts of genetic material, but are also reliant on minimal contamination of the sample. Another technique that has been used to identify PCN is restriction fragment length polymorphism (RFLP). Marshall and Crawford (1987) developed this diagnostic tool for PCN species identification. This technique, combined with PCR, when it is known as amplified fragment length polymorphism (AFLP), is highly sensitive; although also sensitive to contamination it is accurate and can be used for cyst nematode diagnostics.

Enzyme-linked immunosorbent assay (ELISA) relies on specific monoclonal antibodies as probes, which differentiate between species using. It is a relatively cheap and robust method of identifying and quantifying populations of PCN (Davies, Curtis and Evans, 1996).

Ibrahim *et al.* (2001) found that all current identification techniques are laborious but the PCR and IEF results can be obtained in the same day, whereas the ELISA tests take two days. They also suggested that further research was required for the identification of primers and antibodies, for PCR and ELISA analysis respectively.

1.4 Biology and life-cycle of PCN

As with other cyst-forming nematodes, PCN are primarily temperate pests. Potato cyst nematode success is due to their long host-specific relationship with the potato and ability to adapt to environmental changes, robust survival strategies for non-suitable conditions, and high reproductive abilities in favourable conditions.

There are distinct morphological differences between male and females PCN. The males

are vermiform and can move through the soil whilst the females are spherical in shape and are sessile on reaching maturity. It is the mature female body cuticle that at the end of the life-cycle hardens and forms the cyst. This cyst can contain between 200 and 500 embryonated eggs. The life-cycle of PCN can be split into an active and a non-active dormant phase (Turner and Evans, 1998).

In the presence of a host crop and suitable environmental factors the active phase of the life-cycle of PCN occurs (Figure 1.1). The nematodes hatch from the eggs as second stage juveniles (J2), triggered by specific chemicals (hatching factors) released from the host plant roots within the family Solanaceae (Robertson and Forrest, 1989). Under suitable environmental conditions, hatch of more than 80% has been recorded (Fenwick, 1949). In the absence of a host crop PCN will also hatch (see section 1.7.3).

Sense organs in the head (amphids) could possibly be responsible for the movement of J2 along a concentration gradient to locate the root (Turner and Evans, 1998). The J2 uses its sharp stylet to cut its way into the epidermal cells and then through internal cells, leaving a path of ruptured cells (Evans and Stone, 1977). The J2 then feeds through a 'feeding tube', which acts as a filter to large molecules. The J2 injects saliva into the root cells, which then enlarge. Their cell walls begin to break down and a syncytial transfer cell is formed allowing the nematode to obtain nutrients from the plant via the enlarged surface area that connects the transfer cell to the plant's vascular tissue.

Whilst the nematodes are able to get sufficient nutrients from the host plant they will continue to develop at the syncytium; this may take up to 3 months (Jones and Northcote, 1972). The sex of the nematode is undetermined until after infection of the root. If the nematode is unable to obtain enough nutrients from the host it may grow deformed or, since male PCN require 1% of the energy that females require for full development, may develop as a male under the conditions of nutrient stress (Trudgill, 1967).

The males remain coiled until the final moult to the J5 stage, and do not feed after the J3

stage. On reaching maturity in stage 5, the male nematodes are about 1mm in length. At this stage they leave the root and can survive in the soil for up to 10 days (Evans, 1970).

As the females develop they become more bulbous, and increase in size, mainly due to gonad development. On reaching stage 5, the female ruptures the root cortex but remains attached to the root by the head and neck, the females are secured in place by a cement fixative. The female then releases a sex pheromone to attract the male, which fertilises her eggs (Riga *et al.*, 1996). The male can mate several times. After fertilisation the embryos develop to the unhatched J2 stage. The female then dies and her body cuticle hardens and becomes the cyst. The cyst eventually breaks off the plant and remains in the soil.

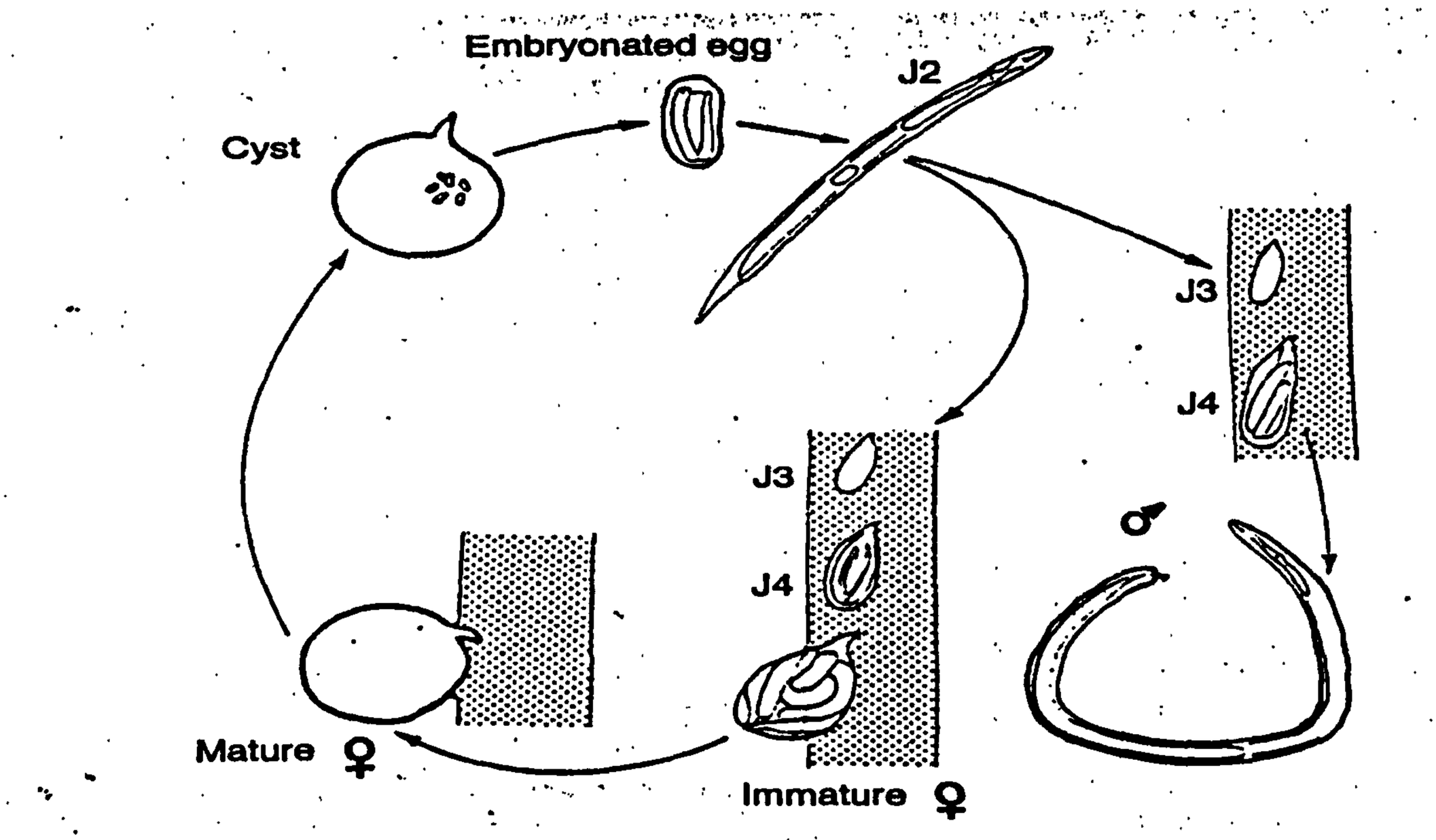


Figure 1.1 Life-cycle of *Globodera* spp. potato cyst nematodes (Evans and Stone, 1977).

1.5 Effect of PCN on host plant

The juvenile invasion and subsequent feeding in the potato plant root system can result in stunting of the plants, decreased development and subsequent lower crop yield.

At very low population densities Jones (1957) found that cyst nematode infestation of

plants could result in increased crop yield. This was attributed to the plants producing more lateral roots in response to attack, resulting in greater yield. At higher infestation levels the yield losses are proportional to the degree of infestation. In the past, 20 eggs g^{-1} soil was used as the economic threshold for a field crop (Evans and Stone, 1977). However, this figure is dependent on a number of environmental factors, such as soil type, PCN species, soil temperature, cultivar grown, and PCN management practices employed.

The effect of PCN infestation at moderate and high levels is to reduce the size of the plant rooting system. The nematodes burrowing into the roots leave tracts of dead and damaged cells, and this is the most important direct damage caused to the potato plant by PCN. The resulting plants are smaller and less developed due to nutrient deficiency (Trudgill, Evans and Parrot, 1975). For this reason infected plants are more susceptible to water stress, wilt easily and are prone to premature senescence with resulting yield losses (Haydock and Evans, 1998).

Additional indirect effects of PCN infection result from the entry lesions on the roots allowing invasion by other organisms. This has been found to be the case for the fungus *Rhizoctonia solani* (Grainger and Clarke, 1963), and the fungus *Verticillium dahliae* (Corbett and Hide, 1971; Evans, 1987). These studies found that when both the fungus and PCN were present in the soil yield losses were greater than when only one of the organisms was present.

The direct effect of PCN on potato yield in the UK is thought to be responsible for losses of around 9% of production (Evans and Stone, 1977). This figure equates to losses of £54.4m based on the mean value of the UK potato crop from 1994-1999 (BPC, 2000). This figure does not take into account the associated costs of potato production, such as nematicides (Haydock and Evans, 1998).

1.6 PCN field infestation

It is important to understand PCN population dynamics within a field and the methods of studying them. The infestation of a field by PCN is not uniform and this results in various problems for quantifying and managing infestations in fields (Been and Schomaker, 1999). This section will consider the modes of infestation and the subsequent spread of PCN within a field.

1.6.1 Initial field infestation

Due to their small size (less than 1mm) cysts can be easily moved. They can arrive in new countries in soil on tubers and even washed tubers can still carry cysts in the eyes and creases of the surface. If the seed tubers for a crop are contaminated by cysts they can become successfully established in a previously un-infested field. However, the main mode of initial infestation appears to be by farm machinery, either in soil on cultivation equipment or on tractor tyres, which results in most initial populations being found at the entrance to fields (Webster and Boag, 1992). Other possible modes of transport are farm workers footwear and hooves of livestock. On contoured land contaminated soil may be washed down the slope and even into other fields. Cysts may also be spread by the wind on sandy soil and highly organic soils. A further method of infestation is the movement of large amounts of soil into a field, such as the soil returned to the land following the washing of potatoes in processing plants.

1.6.2 Within-field distribution

Populations of PCN are found in varying degrees of intensity throughout a field: even a highly infested field will have non-infested areas or areas of low population density. As a result the PCN population density does not show spatial homogeneity in fields (Riding and

Parker, 2000). This is of particular interest for work looking at selective applications of nematicides and when considering methods to quantify nematode population densities by soil sampling. The areas where PCN are found in a field are called infestation foci, with infestations gradually spreading out from these to form secondary foci.

Due to the limited mobility of J2 nematodes, which move often much less than 1 m and cysts being sessile, PCN do not actively colonise un-infested areas. They can however, be moved in soil water or by translocation of soils. Within-field movement of PCN is mainly as a result of cultivation practices (Been and Schomaker, 1996) or by soil erosion down a gradient. Additionally the modes of transport mentioned for initial field infestation can also be responsible for within-field spread.

Following the initial infestation (or infestations) the PCN foci are gradually dispersed in the surrounding areas. These are subsequently spread to new areas (Jones and Perry, 1978). The detection of these foci is difficult due to them constantly being spread by cultivation practices. The PCn populations will multiply during a potato crop in a focus and then be spread out into the surrounding soil. This multiplication and dispersal result in the PCN being below crop damage threshold for the subsequent potato crop. It may take over twenty years before patches of damaged plants are first noticed (Bedi, 1968). This could potentially result in a field becoming highly infested before reaching detection levels. (Been and Schomaker, 2000).

Using intensive soil sampling, it is possible to firstly identify the location of a focus in the field and also its shape. This information can then be used to construct spatial distribution models, which with sufficient information on potential movement of populations within a field, could be dynamic and predictive (Been and Schomaker, 2000). Although the intensity of the sampling and subsequent processing is costly, the use of dynamic spatial infestation models could reduce the need for sampling after an initial map is constructed. Although as yet no spatially dynamic models have been constructed for PCN, some have

1.7 Population dynamics of PCN

This section will consider the factors affecting potato cyst nematode populations (Figure 1.2) and will mainly focus on population decline in the absence of a host crop. To understand these interactions the natural hatching process must be looked at in more detail, including the dormancy state of cysts in the field.

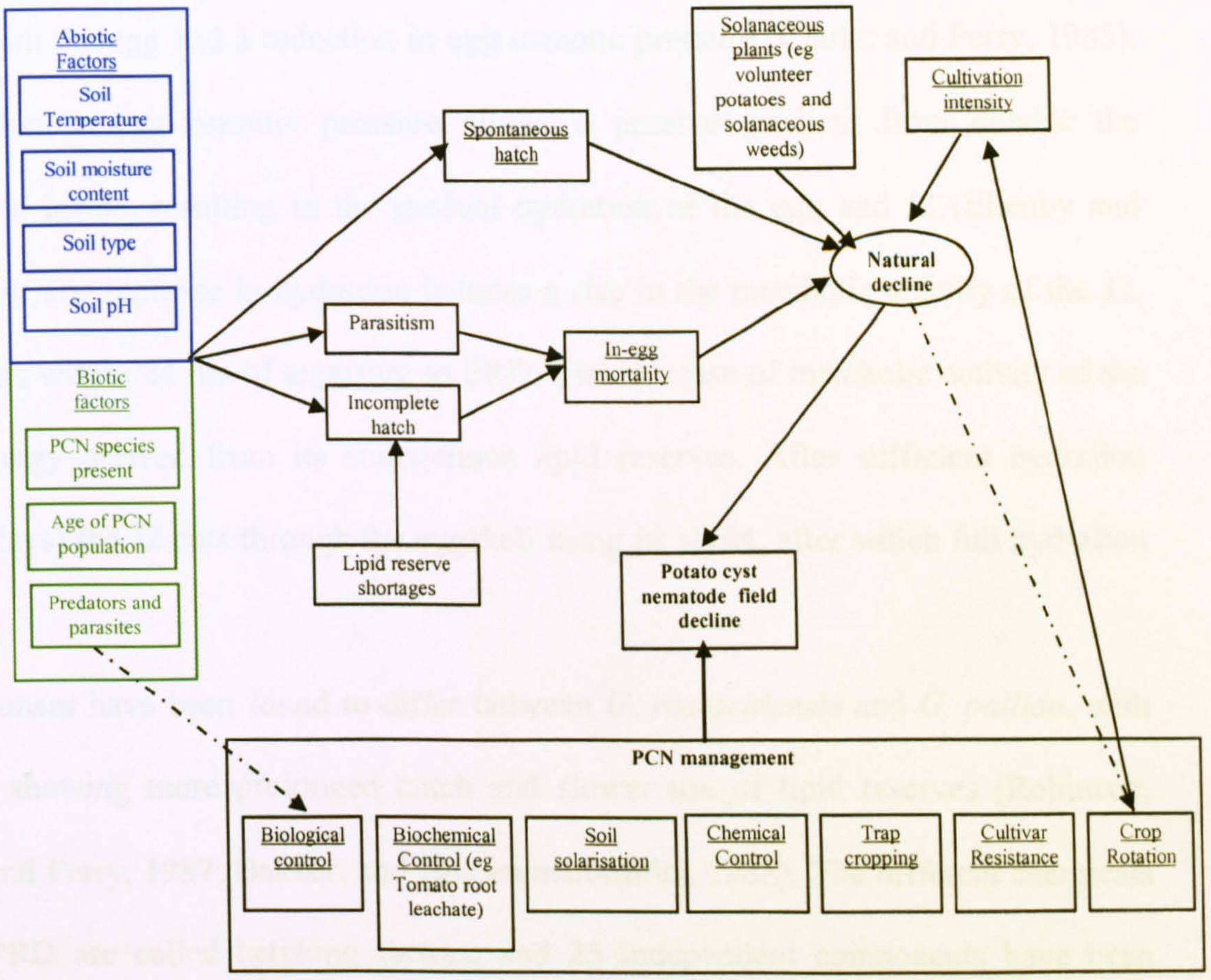


Figure 1.2 Factors affecting PCN field populations

1.7.1 Hatching of PCN

The hatching of cyst nematodes species is strongly influenced by the release of chemicals from the host crop. Potato cyst nematodes, due to the length of their co-evolution with their Solanaceous host, are almost completely dependent on the release of potato root diffusate (PRD) to trigger the hatch of J2 nematodes (Perry, 1987).

It has been found in *in vitro* experiments that exposure of PCN to PRD for several minutes

It has been found in *in vitro* experiments that exposure of PCN to PRD for several minutes triggers the large-scale hatch of the J2 nematodes of *G. rostochiensis* (Perry and Beane, 1982) and *G. pallida* (Forrest and Perry, 1980). This shows that after initial exposure to PRD the concentration does not have to be kept up to ensure subsequent hatch.

Potato root diffusate induces a Ca^{2+} mediated change of structural lipids in the eggshell membrane, resulting in a change in its permeability, which results in the release of trehalose from the egg and a reduction in egg osmotic pressure (Clarke and Perry, 1985). The reduction in egg osmotic pressure allows a passive gradient from outside the membrane to inside, resulting in the gradual hydration of the egg and J2 (Ellenby and Perry, 1976). The increase in hydration induces a rise in the metabolic activity of the J2, which occurs within 24 hrs of exposure to PRD. The increase of metabolic activity of the J2 uses energy derived from its endogenous lipid reserves. After sufficient hydration (around 3 days) the J2 cuts through the eggshell using its stylet, after which full hydration occurs.

Hatch responses have been found to differ between *G. rostochiensis* and *G. pallida*, with *G. pallida* showing more prolonged hatch and slower use of lipid reserves (Robinson, Atkinson and Perry, 1987; Bakker. and Bouwwman-Smits, 1988). The different chemicals found in PRD are called hatching factors, and 25 independent components have been identified (Byrne *et al.,* 1996). Although, hatch by both PCN species has been found to be triggered by these hatching factors, the hatching factors produced by the plant later in its growth cycle were found to cause greater hatch in *G. pallida* than the earlier produced hatching factors. This was not found by Bryne, Maher and Jones (2001), where *G. rostochiensis* was found to have a greater hatch in response to leachate from older plants than *G. pallida.* However, subsequent chemical analysis showed that the plants were producing an increased proportion of *G.* rostochiensis-favouring hatching factors. At least 25 hatching factors have been found to be present in potato root diffusate (Byrne *et al.*,

1996) three such hatching factors for *G. rostochiensis* have been identified as potato glycoalkoids, solanine and α-chaconine (Devine *et al.*, 1996). Byrne, Maher and Jones (2001), using fractionation, placed the hatching factors into distinct groups. They were species selective (active towards both species but resulting in higher hatch of one species than the other), specific (acting on only one species) and neutral (hatching equally for both species). Various abiotic, such as temperature, factors also affect hatch with *G. rostochiensis* having a higher optimal hatch temperature than *G. pallida* (Franco, 1979; Robinson, Atkinson, and Perry, 1987). However, local field adaptations of *G. rostochiensis* have been observed, for example Hominick (1979) observed that other abiotic factors influencing hatch are aeration and moisture (Clarke, Perry and Hennessy, 1977). These will be discussed in more detail as part of factors affecting hatch in the absence of a host crop.

1.7.2 Dormancy

In temperate farming regions such as the UK, only one potato crop is usually grown in a year and, as a result, only one complete PCN generation occurs per year. After fertilisation and cyst formation, the J2 nematodes remain dormant in their eggs. Some egg dormancy may last for years depending on the time until next the host crop is planted. Dormancy has been divided into two forms, quiescence and diapause (Evans and Perry, 1976).

Quiescence is triggered by unfavourable conditions and is broken by the return of favourable conditions. One such factor is the exposure to hatching factors which will break the quiescence and induce hatch (Devine *et al.*, 1996). Diapause seems to be triggered by signals from the host plant in the autumn, which causes dormancy until the following spring. In this state of arrest the J2 can remain unresponsive even to the trigger of PRD, and thus seems to be on biological time lock (Jones, Tylka and Perry, 1998). The differences between these two forms are difficult to discern, especially as quiescence can

follow directly after diapause.

Quiescence is a spontaneous reversible response to unfavourable environmental conditions, which can be activated at any time of year. den Nijs and Lock (1992) observed that *G. pallida* had a greater requirement for PRD than *G. rostochiensis* in order to exit quiescence. This suggests a possible difference in hatching strategy between the species (see section 1.7.1).

Diapause can be further split into two types: obligate diapause and facultative diapause. PCN go through obligate diapause only once in their life-cycle, which in *G. rostochiensis* is directly after the development of the J2 (Antoniou, 1989). It is triggered by various endogenous factors. Facultative diapause is triggered by exogenous factors, and can be terminated by these external factors after a critical amount of time. Initiation of facultative diapause in *G. rostochiensis* can be as a result of photoperiod (Franco and Evans, 1979) and low temperatures (Hominick, 1979). As a result this type of diapause can also be referred to as 'winter dormancy' (Jones, Tylka and Perry, 1998).

The cyst protection afforded by the eggs allows PCN to survive a large number of adverse conditions, such as the drying of their surroundings. Changes in the permeability of the eggshell allow survival of desiccation by limiting the dehydration of the J2. Upon hatching, the J2 nematodes have no mechanisms for survival of desiccation (Ellenby, 1968).

1.7.3 Natural decline of PCN populations

The use of crop rotation is the oldest management strategy for reducing populations of potato cyst nematodes (Haydock and Evans, 1998). It exploits the natural decline of PCN in the absence of a host crop, and an overall decline rate of 30% has been generally accepted in the past (Hancock, 1988). However, the complexities of site interactions and populations mean that the actual decline rate can vary greatly between sites (Table 1.1).

Natural decline can be the result of spontaneous hatching of J2 nematodes, when soil temperature and moisture are optimal. It is dependent on the same external factors as hatch under a host crop (Figure 1.2). Decline is also affected by the presence in the soil of predators or parasites, which may attack unhatched J2 nematodes. A further possible reason for decline is in-egg mortality of the J2 (Devine *et al.*, 1999). In-egg mortality can be due to parasitism or incomplete hatch, resulting in the unhatched or partially hatched J2 nematodes being more vulnerable to environmental conditions (Forrest, 1989).

As with the hatch of PCN under a host crop, the decline rate is affected by site abiotic and biotic factors. As mentioned earlier, the separation of PCN into the two species *G. rostochiensis* and *G. pallida* was only in 1972 (Stone, 1972b), so work carried out before this time does not take into account possible decline rate differences between the two species being present. A further point to note is the method of measuring decline. Some earlier research quantified PCN populations by counting 'full cysts' (Cooper, 1953; Winfield, 1965), which potentially underestimated any decline taking place (Whitehead, 1995). This potential underestimation is due to this method counting any cysts with more than 50 eggs as 'full', whereas cysts can hold up to 500 eggs.

Table 1.1 Review of PCN natural decline rate studies

Author	Year	Experiment Type	Location	Soil Type	PCN species	Previous crop	Initial PCN Population	Site Use/ Rotation	Length	Decline Rate over period (per year unless stated)
Huijsman, C.A.	1957	Field as below	Holland	Sandy	*Heterodera rostochiensis*			beans	2 years	40%
Cole, C. S. and Howard, W.	1959	Field Plots 42 plots (2x2m), 1.5m path between fallow	Little Ouse, Norfolk	Black Fen soil	*H. rostochiensis*	Rye	51 cysts/ 100 g soil and 82 eggs g soil	3 year fallow	3 year	68% duration of experiment
Grainger, J.	1959	Greenhouse and field	Ayshire, Scotland		*H. rostochiensis*			Moisture (%) / Temp. (^{0}C) 20 / 5 20 / 10 20 / 15 20 / 20 20 / 25 14 / 5 14 / 10 14 / 15 14 / 20 14 / 25	1 year	 10% 17 26 41 52 17 29 38 55 65
den Ouden, H.	1960	Field trial 72 squares of 1m^2	Holland	Sandy soil	*H. rostochiensis*		2.1 cysts/ g soil and 109 eggs/ g soil	3reps no potatoes	3 year	60% 53%, 52%, 48% 49% **Mean 52%**
Huijsman, C.A.	1961		Wageningen, Holland		*H. rostochiensis*			beans	5 years	33%
Cole, C. S. and Howard, W.	1962	Microplot (3ft.4in.x 2ft.4in.)	Sandy, Bedfordshire	Sandy loam	*H. rostochiensis*		3.14 cysts/ g soil and 455 eggs/ g s	1.Ryegrass/Red Clover. 2.Barley. 3.Sugar beet. 4. Wheat.	4 year	62% 25% 5% 9% **Mean 25%**
Cole, C. S. and Howard, W.	1962	Field Plots 42 plots (2x2m)	Little Ouse, Norfolk	Black Fen soil	*H. rostochiensis*		51 cysts 100 g soil and 82/ g s	5 year fallow	5 year	24% of initial pop. In 1955

Continued.

Author	Year	Experiment Type	Location	Soil Type	PCN species	Previous crop	Initial PCN Population	Site Use/ Rotation	Length	Decline Rate over period (per year unless stated
Grainger, J.	1964		Ayrshire,Scotland		*H. rostochiensis*			*Tagetes minuta* (estd) ***Tagetes minuta*** (agric) ***Lolium perene***	1 year 1 year 1 year	50% 20% 30%
Winfield, A.L.	1965	Soil survey (25 cores for 10acres, corer 20 x 2.5 cm)	Holland, Lincolnshire		*H. rostochiensis*		0.43 cysts g soil		10 years	50%
den Ouden, H.	1970		Holland		*H. rostochiensis*					20%
Stone, L.E.W. *et al.*	1973	Microplots, 16 (91 x 41 cm). 20 cores (23 cm deep)	Cardiff	Organic sandy loam	*H. pallida*			Arable (non-potato) Cereals Grass	5 years	22% 18% 19%
den Ouden, H.	1974		Holland							49%
Duggan, J.J.	1978	Microplots, 6 x 4 ft. on a field scale	Ireland	Varied soil types	*G. rostochiensis*					30-40% **33% mean**
Storey. G. W.	1984	4 blocks of 6 plots (6m by 9 plots), one fallow	Ormskirk, Lancashire	Peaty loam Sandy loam	*G. rostochiensis* *G. rostochiensis*	Potato		Fallow Fallow	1 year	39% 35%
Wharton, R.J.	1986	Field sites				Previous potato crop	9-78 eggs g soil		2.5 years	
			Humberside	Sandy loam	*G. pallida*	1979				54
				Sandy loam	*G. rostochiensis*	1979				5
				Sandy	*G. pallida*	1976				49
				Sandy	*G. rostochiensis*	1975				62
				Peaty	*G. pallida*	1979				10
				Peaty	*G. pallida*	1978				54
			Lancashire	Clay peat	*G. pallida*	1979				43
				Clay peat	*G. pallida*	1979				50
				Clay peat	*G. pallida*	1979				45
				Clay peat	*G. pallida*	1979				31
				Clay peat	*G. pallida*	1975				59
				Clay peat	*G. pallida*	1979				40

Continued.

Author	Year	Experiment Type	Location	Soil Type	PCN species	Previous crop	Initial PCN Population (per g soil unless stated)	Site Use/ Rotation	Length	Decline Rate over period (per years unless stated)
Whitehead, A.G., Webb, R.M. and Beane, J.	1991	Field plot	Bedfordshire	Sandy Loam				6 barley	6 year	51.5
Whitehead, A.G.	1995	Microplots 9 circular microplots (90cm dia x 25cm depth)	Rothamsted							
			Methwold Hythe	Peaty loam	*G. rostochiensis*	Potato	14.4 cysts 941 eggs	Spring Barley	4 year	61%
			Woodwalton	Peaty loam	*G. rostochiensis*	Potato	9.0 cysts 638 eggs			71%
			Ramsey	Peaty loam	*G. rostochiensis*	Potato	4.9 cysts 255 eggs			48%
			Carlton	Medium loam	*G. rostochiensis*	Potato	4.5 cysts 418 eggs			58%
			Butterwick	Medium loam	*G. rostochiensis*	Potato	4.3 cysts 292 eggs			68%
			Woburn	Sandy loam	*G. pallida*	Unknown	2.5 cysts 75 eggs			45%
			Trumpington	Medium loam	*G. pallida*	Unknown	0.6 cysts 22 eggs			69%
			West Fen	Peaty loam	*G. pallida*	Unknown	0.6 cysts 69 eggs			52%
			Mepal	Peaty loam	*G. pallida*	Unknown	4.2 cysts 47 eggs			86%
Turner, S.J.	1996	Field samples 20 sub samples (1.7x 8cm auger), 4 per field 0.25ha then 2 per 0.25ha up to22 for 2.5ha.	Northern Ireland					varied	13 year	17 years 98.7% decline, average 50%
Devine, K.J *et al.*	1999	Pot expt 6 replicates from 6 plots at 2 field sites (9 cores 2.5 cm diameter and 30 cm depth samples bulked)	County Cork, Ireland	Site A	*G. rostochiensis*	Potato		Fallow	May to Septem-ber (20 weeks)	57% decline of viable eggs
				Site B	*G. rostochiensis*	Fallow (potato previous year)		Fallow		30.3% decline of viable eggs

Although *G. rostochiensis* and *G. pallida* are closely related sibling species that occupy the same ecological niche, they use different strategies to ensure their survival and population increase. *G. pallida* reacts mainly to the release of PRD as a hatching trigger and, in so doing synchronises its life-cycle with that of the host plant (den Nijs and Lock, 1992). This agrees with the findings of Robinson, Atkinson and Perry (1987), who found that this species showed a slower initial hatch and use of lipid reserves than *G. rostochiensis*. As an ecological strategy, slower hatching would avoid over-infestation of younger roots and allow root development, thus increasing the potential number of infestation sites and reducing intra-specific competition. In contrast to this the *G. rostochiensis* shows a more opportunistic approach, in that it relies less on PRD as a hatching stimulus; the presence of other factors, such as suitable soil temperatures, are sufficient (den Ouden, 1960), which means that the potential for hatch in the absence of a host crop is greater. This may lead to the populations of *G. rostochiensis* having a higher decline rate in the absence of a host crop. Hatched J2 nematodes of *G. rostochiensis* have been found to survive in the soil for over seven weeks, which was longer than *G. pallida* under the same conditions (Mulder, Pieterman and Vroom-Wolf, 1988). The result of this may lead to greater success of the J2 nematodes of *G. rostochiensis* than that of *G. pallida* in the years when potatoes are planted on a site.

The interactions of different organisms within soil are complex. PCN attacks and parasitises plants of the potato family but it is also attacked in turn by other organisms, which may be parasites or predators, among which are species of fungi, bacteria and other nematodes. The relationships between parasites and predators of PCN have been widely studied as a means of biological control (see PCN management). The natural presence of these organisms within a field will affect the decline rate of PCN (Kerry, 1987).

In order to hatch, PCN require adequate surrounding moisture, and can survive in a state of quiescence in dry soil, due to osmotic stress (Dropkin, Martin and Johnson, 1958; Wright

and Newall, 1976). To allow spontaneous hatch to occur the soil moisture level must be sufficient for the osmotic gradient into the J2 through the eggshell membrane to allow hydration of the juveniles and their subsequent increased activity (Clarke, Perry and Hennessy, 1978). Oostenbrink (1950) observed a decline rate of 20% in dry soil and 50% in soil kept at a moisture level sufficient for *H. rostochiensis* hatch, which seems to agree with the need for moisture to allow hatch, although, the exact moisture contents used for this work are not recorded. However, Grainger (1959), in studies looking at the effect of temperature and moisture on *H. rostochiensis*, found that for all temperatures tested (5°C - 25°C), the decline rate was significantly higher with a 14% total soil moisture content than at 20%. However, a moisture content of 14% in UK soil is not drought level so PCN hatch would probably not be impeded by it.

The growing of non-host crops seems to result in differing rates of PCN decline. Cooper (1953) showed greater *H. rostochiensis* decline rates under arable crops than under grassland, although this may be more due to the effects of bulk density, with the PCN density being greater in soil of greater in soil bulk density. When a field with *G. rostochiensis* present was left in bare fallow the decline rate was 20% (La Mondia and Brodie, 1986). The relationship between crop and decline rate was not seen in other work carried out using pots and microplots. Cole and Howard (1962) found no significant difference in decline under barley and ryegrass, with 36% and 38% decline rates respectively, for *H. rostochiensis*. This was also found by Whitehead (1995), who found decline rates for both species (20% for *G. pallida* and 22% for *G. rostochiensis*) well below the 33% average decline found by Cooper (1953). In 1973, Stone *et al.* carried out work on *G. pallida* to examine differences in decline between cropping regimes over 5 years and found little difference (general arable 22%, horticultural arable 19%, cereals 18% and grass 19%). Since microplot experiments did not show similar trends, they concluded the reason for the differences in decline between grass and arable crops is

probably due to the ploughing speeding up the decline, as opposed to direct effects of the crops (Whitehead, 1995). PCN cysts in heavily cultivated soils may be damaged by the mechanical operations. Also the cultivation practices will spread out the PCN population on a site, resulting in samples collected from that location having a lower population density after each cultivation practice.

Differences in decline rates have been observed in populations from fields with different soil types. Cooper (1953) stated that, after a crop failure due to PCN, a rest of five years is necessary for heavy soil and about ten years for lighter soils. Observations made by Cole and Howard (1962 a; 1962 b) contradict this suggestion of different decline rates for soil types; decline rates in black fen, peaty and silty soils was around 33%, whereas for sandy soils the decline rate was 60%. Both these experiments were looking at *H. rostochiensis*, which could mean that they were looking at declines of different species. Also the age of the cysts is not mentioned, and this could have a major influence on decline. Stone *et al.* (1973) found that, on sandy loam, a *H. pallida* population had a decline of 20%. In the work carried out by Whitehead (1995), there is evidence that soil type does influence PCN decline. *G. rostochiensis* population decline varied with soil type (silty-loam 12.8%, medium-loam 20.1% and peaty loam 45%). For the *G. pallida* populations in the same experiment there was no similar relationship (medium-loam 18.9% and peaty-loam 21.6%).

Soil temperature is a key factor in the hatching of PCN. Average soil temperatures, during the growing season in the UK, range between 15 and 18°C, which is similar to those found in the potato growing areas of South America. Franco (1979) reported that PCN hatch occurs between 8 and 27°C, with *G. rostochiensis* having an optimal hatch temperature of 20°C and *G. pallida* of 16°C. The two species also vary in their minimum hatch temperature, with *G. pallida* able to hatch at 8°C and *G. rostochiensis* 10°C. This difference may be a central reason for the increase in the proportions of *G. pallida* in UK

field populations, with *G. pallida* able to exploit early planted potatoes at lower spring temperatures. However, Hominick (1979) was able to show that *G. rostochiensis* can adapt to hatching in cooler soils. This was proved by continually growing early potatoes on a field infested with *G. rostochiensis*, which adapted to the early spring temperatures to hatch with the potato crop. In work carried out by Devine *et al.* (1999), soil temperature affected spontaneous hatch of *G. rostochiensis*, with greater hatch at 20°C (36%) than at 4°C (2%).

The age of the cysts is also thought to be a factor involved in the rate of decline of PCN, with the initial year after cyst formation showing the highest level of hatch. This was suggested by den Ouden (1960), who found an initial decline rate for the year after the last host crop of 50% and only 36% for the subsequent year for *H. rostochiensis*. Turner (1996) found in a survey that the average decline of field populations in the year after a potato crop was 50%. Huijsman (1957) found, over a two-year period following potato crop declines of 34% then 11%, for *H. rostochiensis*. A study of two fields with *G. rostochiensis* by Devine *et al.* (1999), one in the first year after potatoes and the other in the second, found declines of 57% and 40.3%. Within these decline rates, egg mortality was responsible for 11.3% and 9.3% respectively. An initial higher decline rate was also found for *G. pallida*, with an initial decline of 25% and subsequent decline of 8% (Stone *et al.* 1973).

1.8 PCN management

Due to the potential for economic losses from a potato crop caused by PCN there has been extensive study into possible measures for control. As mentioned previously it is important to understand the specifics of the site and PCN population dynamics. These specifics include the PCN species composition, the population size, its distribution and its soil type and temperature. Once a clear picture of the field's infestation and site factors has been

achieved the control methods can be selected.

The object of PCN management is two-fold: short term management for the prevention of yield and quality losses of the next potato crop and long term management to maintain the PCN population of the field at a level where it is economic in subsequent years to grow potatoes. This requires maintenance of the PCN population around the damage threshold (for a susceptible cultivar usually around two eggs g^{-1} soil) (Whitehead and Turner, 1998).

It is important when determining a PCN management protocol to understand the population multiplication rate, which is inversely proportional to density, i.e. it is greater at low population densities prior to potato crop (Peters, 1961).

To achieve successful PCN management in a field it is normally necessary to implement two or more control measures. Currently used and developing control measures include crop rotations, resistant/tolerant cultivars, nematicides, trap cropping, soil sterilisation and biological control. These are combined in integrated control strategies, whose effects can be modelled in predictive PCN management computer simulations (Trudgill *et al.*, 2003).

1.8.1 Crop rotation

This method rests the field from the potato crop, for a sufficient number of years to allow natural decline of PCN to economically viable levels to take place. This is the earliest recorded method of PCN control with the earliest known example being a seven-course rotation being implemented by the Incas in S. America to avoid "potato sickness" (Haydock and Evans, 1998). Given the life-cycle in PCN, growing a susceptible cultivar for consecutive years will lead to strong multiplication of both *Globodera* spp., resulting in heavy infestation levels and diminished crop returns. PCN can only multiply in the presence of certain solanaceous hosts (potato, aubergine and tomato). If the field is devoid of host plants then no multiplication occurs but spontaneous hatch and egg mortality do. Thus, the success of crop rotation is dependent on the natural decline rate of the species on

the given site. It is also important to remove any potential host plants from the field in the rotations following a potato crop in order to achieve the maximum PCN decline. These may be volunteer potatoes (viable tubers left in the soil from the previous crop) and solanaceous weeds (den Ouden, 1967).

Whitehead and Turner (1998) used a formula to predict the length of rotation required to return the PCN population of a field to below damage threshold levels (Figure 1.3). This formula assumes a density independent decline.

$$n = \frac{Pi - Pf}{Pc}$$

Figure 1.3 Prediction formula for rotation length required for control of PCN in a field. Pi= initial population (number aimed for); Pf= population post-crop (population after last potato crop); Pc= fraction of eggs left in cyst after one year (from decline rate); *n*= number of years (Whitehead and Turner, 1998).

Using this formula in conjunction with the range of decline rates mentioned earlier (Table 1.2) provides examples of the number of years of non-host crops required to lower the PCN population to below the economic threshold (5 eggs g^{-1} soil) for a potato crop.

Table 1.2 Number of years of non-host rotation to return PCN population to economic levels.

Decline Rate (% per annum)	Return population to 5 eggs g^{-1} soil			Return population to 15 eggs g^{-1} soil		
	50 (eggs g^{-1} soil)	100	200	50	100	200
60	2.5	3.3	4	1.3	2.1	2.8
40	4.5	5.9	7.2	2.4	3.7	5.1
20	10.3	13.4	16.5	5.4	8.5	11.6

The decline rate is crucial when selecting the length of rotation. With a decline rate of 30% per annum over 5 years without a host crop, the PCN population will decline by 83%, however, a decline rate of 10% over the same period results in a reduction of only 41%. For this reason, crop rotation has large variations in its potential effectiveness for PCN control. The economic pressure on the grower to reduce rotation length due to other crops tending to be of lower value is also a central pressure in the use of crop rotation as the sole management method employed (Haydock and Evans, 1998). More information on the natural decline rates of PCN populations is needed to evaluate properly the length of crop rotations that must be combined with other management practices.

1.8.2 Resistant/tolerant cultivars

There is much variation in the extent to which cultivars allow PCN to multiply on them. Fully susceptible cultivars allow nematodes to freely multiply on roots and stolons; in comparison fully resistant cultivars allow no nematode multiplication. The levels of resistance within cultivars vary within the extremes of fully susceptible and resistant.

Resistant and susceptible cultivars are invaded equally by J2 nematodes, which establish feeding sites. The difference, however, is that the feeding sites in the resistant cultivars break-down, not allowing the PCN enough food to develop into a mature female (see section 1.4) and so reducing the potential for population multiplication. A single major gene (H1) provides resistance to *G. rostochiensis* (Howard, 1969), and is widely included in new cultivars by plant breeders. Initial use of this resistance gene resulted in decline in *G. rostochiensis* populations of more than 80%. In the UK, there appear to be no *G. rostochiensis* populations able to overcome the H1 gene. However, the use of these cultivars (especially Maris Piper) has increased population of other resistant *G. pallida in the UK.* For *G. pallida*, only partially resistant cultivars are currently available and these reduce multiplication but do not prevent it. Partial resistance also selects for the pathotypes

that are most virulent to the cultivar. A further problem is that these cultivars tend to be less acceptable to consumers and, therefore, growers (Haydock and Evans, 1998).

Cultivars are separated by their abilities to withstand the attack of PCN into tolerant and intolerant. Tolerant plants are those defined as plants able to withstand attack by a pest without suffering serious damage (Trudgill, 1986). Tolerant cultivars allow potatoes to be grown on moderately infested fields. This is important as even if a crop is resistant it may not survive heavy attack. Tolerant cultivars can be used on shorter rotations but their too frequent use will result in population increase to levels they cannot tolerate (Whitehead and Turner, 1998). Tolerance and resistance are independent, but resistance can produce tolerance in a cultivar.

1.8.3 Nematicides

Two types of pesticide are used for PCN control: soil fumigants and granular nematicides. The soil fumigants are injected into the soil, the surface is sealed and the gas then spreads through the soil profile. It can be applied at any point during a rotation when no crop is present and soil conditions are suitable. The fumigant works best in moist, well-drained soil with low organic matter and low clay content. The viability of populations of PCN in a field under ideal conditions for application can be reduced by up to 80%. However, if after injection the soil surface is covered with plastic sheeting, the decline rate can be higher as the fumigant remains in the soil for longer (Whitehead, Fraser and French, 1979). The fumigant normally used in the UK is Telone® (1,3- dichloropropene); this chemical can permeate the cyst and kill the eggs and juveniles inside (plastic sheeting is not used with this fumigant).

The granular nematicides are applied before potato planting and are applied in smaller doses than the fumigants. The three main granular nematicides used in the UK are Vydate® (oxamyl), Temik® (aldicarb) and Nemathorin® (fosthiazate). Granular

nematicides dissolve in the soil water and work by paralysing and disorientating the hatched J2 nematodes thereby reducing the number that reach host plants and are able to invade. Granular nematicides can be highly effective at controlling PCN populations, especially *G. rostochiensis*, which hatches over a shorter time frame after planting. In 1976, Moss, Crump and Whitehead found granular nematicides were effective at controlling mixed populations of PCN. However, delayed and prolonged hatch of *G. pallida* means that the majority of *G. pallida* J2 do not hatch until after the chemical has dissipated (Whitehead, 1992), and the granular nematicides do not permeate the cysts or eggs (Evans, 1993). Whitehead, Nichols and Senior (1994) found that granular nematicides could control *G. pallida* populations in sandy loam soil, but had negligible effect on populations in peaty loam soil.

The application of nematicides tends to be limited to high value crops due to their relatively high costs, with fumigants costing approximately £550 ha^{-1} and granules approximately £360 ha^{-1} (Evans *et al.*, 2003). Other considerations are their toxicity to the operator and as environmental pollutants (Evans and Haydock, 1998).

1.8.4 Trap cropping

Trap cropping consists of growing a potato crop for long enough to trigger PCN hatch and invasion, then harvesting the crop before the females fully mature this results in population decline. Webley and Jones (1981) showed that when they planted potatoes in March and harvested 81 days later, populations of both PCN species had declined. This strategy requires the use of a fast establishing and large rooting cultivar, which is harvested after 6-8 weeks. The accuracy of the lifting date can be improved by close monitoring of the accumulated soil temperature to predict hatch (Halford, Russell and Evans, 1998). Without close monitoring and timely lifting this method can result in multiplication of PCN.

Trap cropping can be highly successful at controlling *G. rostochiensis* with field trials

resulting in 80% population decline, but is generally less effective at controlling *G. pallida* due to its longer hatch period (Webley and Jones, 1981). However, Whitehead (1992) found that in heavily infested soils *G. pallida* populations could decline by 75%.

1.8.5 Soil solarisation

This method can be used in hot climates or in glasshouses. It has been used in horticulture by artificially heating the soil (electrically) to levels that kill nematodes. In fields covered with clear polyethylene film sheets, the sun can heat the soil to levels sufficient to kill nematodes in the top 20 cm. La Mondia and Brodie (1984) found that using plastic sheeting during a hot summer in the USA resulted in a 98% decline in the *G. rostochiensis* present in the soil to a depth of 20 cm. However, far fewer nematodes were killed during the following cooler summer. The cost of this method for materials and implementation makes it relatively impractical on a large scale. Also, achieving a high decline in PCN levels requires suitable day-length in relation to temperature (Whitehead, 1998).

1.8.6 Biological control

Cyst nematodes have many natural antagonists. The use of these as a method of control is seen as an alternative to the use of nematicides, due to the potential of using the same application equipment. Extensive research has considered the potential of fungi (Kerry and Crump, 1977; Kerry, 1987) and bacteria (Stirling, 1988; Crump, 1989; Sikora and Hoffmann-Hertgarten, 1994). Problems have been encountered trying to convert *in vitro* success to field situations. This is due to problems with production of sufficient quantities of the agent, incorporation of the organism into the soil and its subsequent survival. Crump (1998) suggested that the use of combinations of agents might overcome these problems.

In the Philippines, considerable work has been carried out on the nematophagous fungus *Paecilomyces lilacinus* (Davide, 1995; Davide and Zorilla, 1995), and this has also

encountered the problems mentioned above and so requires further research. In the UK, the fungus *Verticillium chlamydosporium* has potential for controlling PCN. It attacks healthy females on the potato roots, the females then shrivel and their cuticle darkens. This reduces the PCN population, and results in more fungal chlamydospores being produced (Evans and Haydock, 1998).

1.8.7 Integrated control and predictive modelling

Potato cyst nematodes are best controlled when two or more management practices are used together. The over use of individual control measures and shortening of rotations has resulted in the promotion of *G. pallida* in mixed species populations and in an increase in the incidence of virulent PCN pathotypes. One reason for this over use is the lack of knowledge of differences between PCN species/pathotypes and the effectiveness of management practices against them (Evans and Haydock, 2000).

This situation can be rectified by using site and species-specific information to construct Integrated Crop Management (ICM) protocols, which aimed to optimise control while reducing the reliance upon nematicides. Knowledge gained from sampling and construction of a spatial PCN distribution map may eventually allow control measures to be applied in a site-specific manner. This will require more intensive sampling than is currently carried out on commercial sites, which is costly (Elliott, Phillips and Trudgill, 2000). However, savings in nematicide inputs would be possible with better field PCN population maps. It has been pointed out, however, that there is a possibility of missing patches of PCN with application technology and that this could cause future problems, especially as PCN multiplication is inversely proportional to population density (Evans *et al.*, 1998)

Predictive computer models would be important tools for ICM, able to predict PCN damage and multiplication on a site if different management practices were used (Evans

and Haydock, 2000). Building a spatial element into the model has the potential for predicting changes in within-field PCN distribution under a given management regime (Yang *et al.*, 2000). However, as with all computer programmes, the predictive model is only as good as the data it is supplied with (Elliott, Phillips and Trudgill, 2000). In 2001, Trudgill, Elliott and Phillips stated that sampling of the pre and post-crop populations must be improved to increase the accuracy of data collected, which must then be incorporated into the computer programme.

A computer model has been designed to look at integrated control of PCN; this model has been used to look into the potential threat of *G. pallida* in Britain (Trudgill *et al.*, 2003). The model was run altering the approaches to management such as cultivar resistance and nematicide applied. This model is based on data from field experiments to predict future trends but is dependent on some assumptions for which there is insufficient data. The need for individual site-specific data will probably be required to use the computer model to construct an integrated control method for a given site.

1.9 Thesis aims and structure

1.9.1 Aims

The aims of this thesis are to suggest a standard method for monitoring the decline of Potato cyst nematode in the absence of a host crop and to determine the extent of variation in the decline of Potato cyst nematode field populations using a standardised method. Also, to quantify the effect of cultivation machinery commonly utilised within a potato rotation on the movement of Potato cyst nematode.

1.9.2 Hypotheses

The theses tested two hypotheses:

- There are large variations between the natural decline rates decline of Potato cyst nematodes populations, in the absence of a host crop.
- Cultivation machinery utilised within a potato rotation result in the spread of Potato cyst nematodes in field soils.

1.9 Structure

This thesis is split into two sections and structured so that each section can be read in isolation (Figure 1.3). The thesis starts with a general introduction of PCN with a strong reference to research on PCN population decline rates, followed by the thesis aims and structure. Section One examines the natural decline rate of PCN in the absence of a host crop. This section contains two separate methods for monitoring PCN decline (Chapter 2). Section Two examines the movement of PCN by cultivation. Chapter 3 provides a review of the literature on cultivation machinery and soil movement by cultivation research. Chapter 4 examines the lateral movement of PCN in beds by bed-tilling and harvest operations. The movement of PCN cysts in fields by cultivation operations is examined in

Chapter 5. Each experimental chapter starts with an introduction outlining the main points of the chapter. Chapter 6 is a discussion of the main findings from each chapter. All references are located at the end of the thesis.

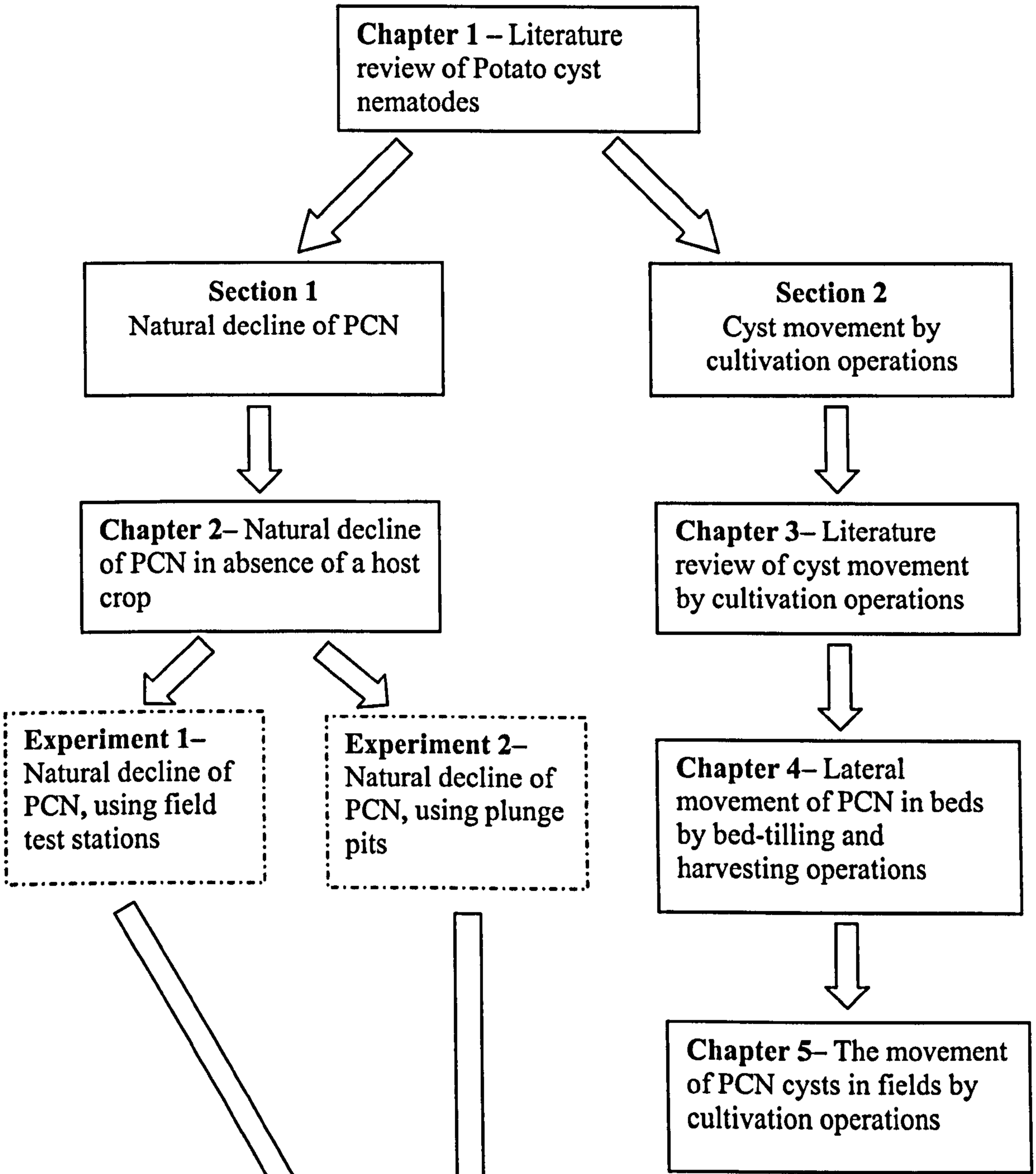


Figure 1.3 Thesis structure.

Section I

Natural decline of PCN

Chapter 2

The natural decline of PCN in the absence of a host crop

2.1 Introduction

This chapter investigates the natural decline of PCN field populations in the absence of a host crop. A number of studies have investigated the decline of PCN (discussed in Section 1.7.3) and have reported highly variable rates, ranging from 5 to 69 % (Table 1.1). Direct comparison between these studies is problematic due to differences in factors such as sampling methods, experimental design, soil type and crop rotational history.

The aims of this chapter were to suggest a standard method for monitoring the decline of PCN in the absence of a host crop; and determine the extent of variation in the decline of PCN field populations using a standardised method. Two different sampling methods were tested to determine the rate of decline. Firstly, the creation of non-permanent test stations within infested fields to monitor in-field decline. The second method removed of field soil from infested fields and placed it in plunge pits.

2.2 General methods

2.2.1 Soil texture analysis

Soil texture analysis was carried for all the fields in the decline studies using methods outlined by Rowell (1994). Where more than one location in a field was sampled separate soil texture analyses were carried out.

2.2.2 Identification, quantification and species determination for PCN

The number of cysts and viable eggs were estimated using the methods outlined by Southey (1986). Soil samples were dried at 25 °C for 4 to 5 days in a drying room. Dried soil samples were sieved (4 mm gauge) and mixed thoroughly before a 200 g sub-sample was taken. The cysts in the sub-sample were then extracted by water flotation using a Fenwick can (Fenwick, 1940). The resulting cysts and soil residuals were then put through a further flotation process using a conical flask. The resultant extracts were dried for 24 hours in a drying room at 25 °C. The extracts were then examined under a microscope (x

20 magnification) and the number of PCN cysts counted. The first eighty cysts were removed from each sample (fifty for egg counts and thirty cysts for species determination). Fifty of these cysts were soaked in distilled water for 7 days. The water was then removed and replaced for a further 7 days with 0.05 % Meldola blue stain (Ogiga and Estey, 1975). The stain was then removed and the cysts washed thoroughly in water and soaked for 24 hours. The cysts were then crushed and rigorously mixed with 50 ml water. From this suspension a 1 ml aliquot was examined under a microscope (x 40 magnification). The number of viable eggs and juveniles were counted. The Meldola blue stain is absorbed into dead nematode tissue. Partially and totally stained individuals were counted as non-viable, as they were dead or dying.

Using the equation below, the number of viable eggs g^{-1} soil can be calculated.

$$\text{Viable eggs g}^{-1}\text{ soil} = \frac{\left(\left(\frac{\text{Water in egg suspension (ml)}}{\text{No. of cysts used for egg coun}}\right) \text{x No. eggs in 1 ml}\right) \text{x No. of cysts in cyst count}}{\text{Weight of soil sample used (g)}}$$

Determination of species present in each soil sample was undertaken using polymerase chain reaction assays (PCR). DNA of the thirty cysts was homogenised, extracted, amplified and digested using the methods and primers used for commercial analysis by The Plant Health Clinic at Harper Adams University College (S. Edwards, Pers. Com.).

2.3 Experiment 1: Natural decline of PCN in the absence of a host crop, using field test stations

2.3.1 Introduction

The aim of this experiment was to determine whether test stations within a field could be used to monitor PCN decline rates in the absence of a host crop. This experiment was carried out using non-permanent test stations within infested fields. Fields were selected on the basis that they had a potato crop in either 1999 or 2000 and were known to be infested with PCN. The fields were in the locality of Newport, Shropshire. Five fields were selected for the purpose of this experiment, two fields had a potato crop in 1999 and the other three in 2000. The experiment was carried out over a four year duration, with sampling of the test stations in March each year. Field site information including soil type, rotation and cultivations for the duration of the experiment is provided in Table 2.1.

Due to variations found in the PCN populations within the test stations in the first year additional sampling took place in 2001. The aim was to determine whether the variation within the test stations was due to inconsistencies during sample processing.

Table 2.1 Field site information.

Field	Soil type	Crop Rotation (From 1999)	Cultivations
1	Loamy sand	Potato	Plough (30 cm), bed formed, bed tilled, destoned, planted and harvested
		Spring barley	Plough, rotared and drill
		Calabrese	Plough, rotared and drill
		Sugar beet	Direct drill
		Leeks	Plough, power harrowed and drill
2	Loamy sand	Potato	Plough, bed formed, bed tilled, destoned, planted and harvested
		Spring barley	Plough, rotared and drilled
		Winter wheat	Ploughed, rotared and drilled
		Sugarbeet	Direct drilled
		Carrots	Plough, bedformed, destoned, rotavated and planted
3	Sandy loam	Spring barley	Ploughed, rotared and drilled
		Potato	Ploughed, bed formed, bed tilled, destoned, planted and harvested
		2 x Winter wheat	Ploughed, rotared and drilled
		Sugarbeet	Ploughed, rotared and drilled
4	Loamy sand	Winter wheat	Plough and press, combination drill with rotare
		Potato	Ploughed, Triple K x2, rotared x2, planted and harvested
		Spring barley	Chisel ploughed x2, ploughed, pressed and drilled
		Winter wheat	Plough and press, combination drill with rotare
		Sugarbeet	Plough and press, rotared and drilled
5	Sandy loam	Sugarbeet	Ploughed, rotared and drilled
		Potato	Ploughed, bed formed, destoned, planted and harvested
		2 x Maize	Ploughed, power harrow and drilled
		Grass	Plough, power harrow x2 and drilled

2.3.2 Materials and Methods

Each field had three test stations (replicates). A test station consisted of a grid made from polypropylene string (a grid of nine cells, each cell 2 m^2). The location of the test stations within the field was selected at random, excluding 20 m from the field boundary. The test station location's was recorded by triangulation from two permanent posts set in the field boundary. The location of the test station corners were also recorded using a Global positioning system (GPS). In each cell 20 cores (using a cheese type corer, 20 cm depth by 2 cm diameter) were systematically collected in a regular grid pattern to form a bulked sample. A larger single core, using a cheese type corer (30 cm depth by 4 cm diameter) was taken from the centre of each cell to form a second sample per cell. In addition, soil from outside the test station was placed in a plunge pit in the field boundary (30 cm diameter by 30 cm depth). A sample was taken from the plunge pit (1 core, 30 cm depth by 4 cm diameter). Nineteen samples were collected from each test station (Figure 2.1). The fields were sampled annually in the spring over four years, with the initial sampling in March 2000. The fields with potatoes grown in 2000 were sampled prior to the planting of the potato crop. The test stations were monitored on a regular basis for volunteer potatoes after the potato crops. Any found were removed from the test stations and surrounding area.

The additional sampling of the test stations in 2001 was carried out after the standard samples for the year had been taken. A cell within each test station grid was randomly selected for intensive sampling. The 2 m^2 cell was sub-divided into four 1 m^2 sub-cells. In each sub-cell 20 cores (20cm depth by 2cm width) were systematically collected and bulked. Eighty cores from the 2 m^2 cell were then systematically taken and bulked. This produced 5 samples for each of the 15 test stations.

To quantify the populations and carryout speciation the samples were processed by standard methods described in section 2.2.

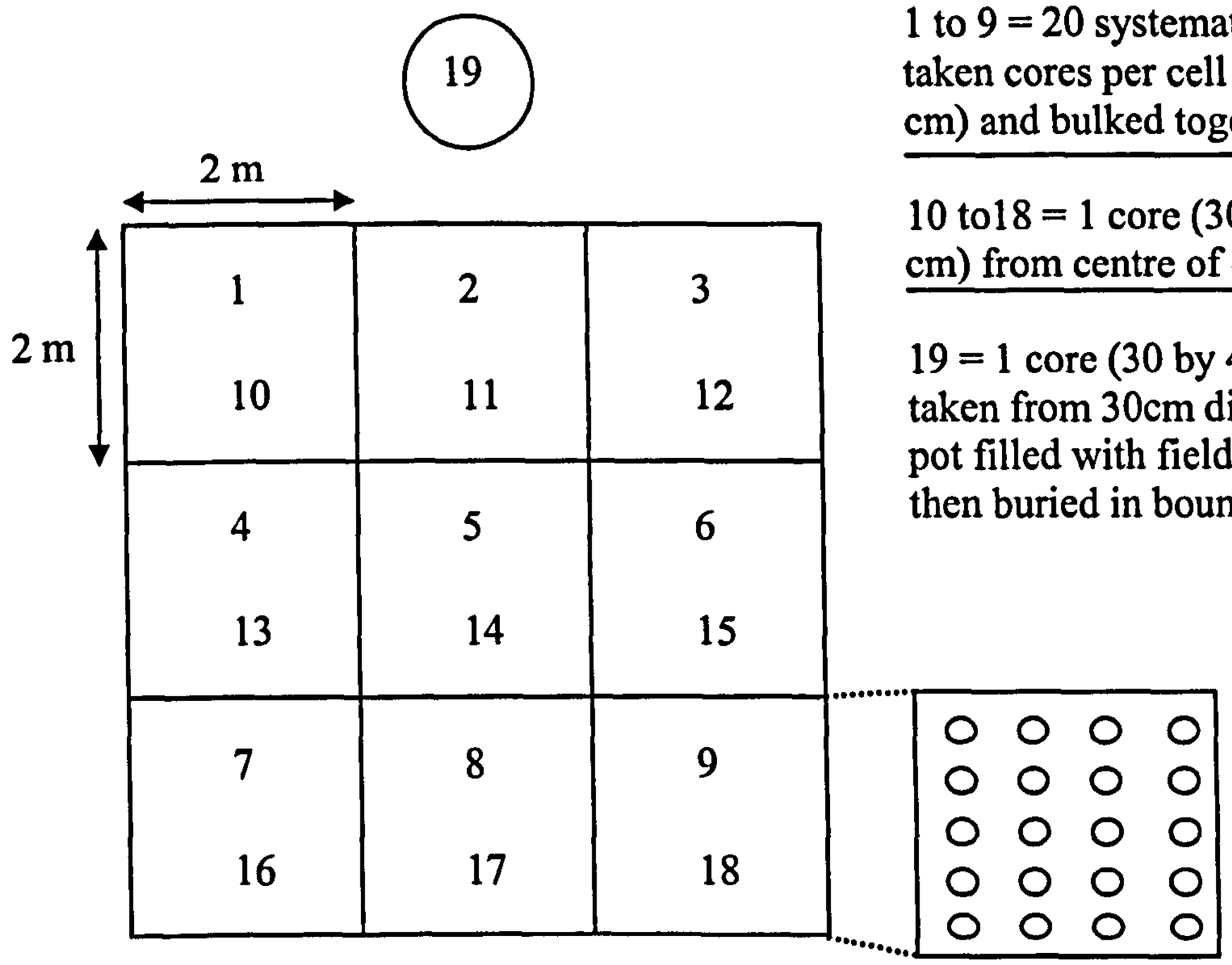


Figure 2.1 Experimental field station design and sampling method.

2.3.3 Data analysis

Data from the five fields were analysed using a General Linear Model (GLM) for multiple analysis of variance and Pearsons Correlation. The results from the additional sampling in 2001 were analysed using a paired T-test. All analysis used the statistical software Mintab 12 (MINITAB INC.).

2.3.4 Results and discussion

PCN Species Identification

Following PCR identification the fields were found to contain differing populations of PCN. Two of the fields had pure populations of *Globodera pallida* in all their test stations, one field had pure *G. rostochiensis* populations in the test stations and the other two had mixed populations (Table 2.2). Both fields with pure *G. pallida* populations in the test

stations had a potato crop prior to sampling, the remaining fields having a potato crop between the first and second sampling years.

Table 2.2 Species present in experimental test stations.

Field	Test station and species present		
	1	2	3
1	*G. pallida*	*G. pallida*	*G. pallida*
2	*G. pallida*	*G. pallida*	*G. pallida*
3	*G. rostochiensis*	*G. rostochiensis*	*G. rostochiensis*
4	Mix	Mix	Mix
5	Mix	*G. rostochiensis*	Mix

Variation within the field test stations

The results of the samples collected in 2000 showed (Figure 2.2) large variation within the standard errors for some of the tests stations. The variation was between the PCN counts for the two sampling methods and between adjacent cells.

The comparison between the sub-division samples and the total area samples are shown in Table 2.3. No significant difference was found between the mean sub-division samples and those of the total area samples ($p= 0.217$). This suggests that the variation observed between the samples in the test stations was not due to inconsistencies during sample processing. Therefore, any variations found in the test station, both spatially and temporally, can be attributed to the differences in PCN population dynamics within the field soil.

These results also suggest that the mean PCN population derived from the cells in a test station grid will be representative of the overall PCN population within the test station. The mean population of PCN, found within the test station, can thus be compared over time to determine the changes in the PCN population dynamic in the test station.

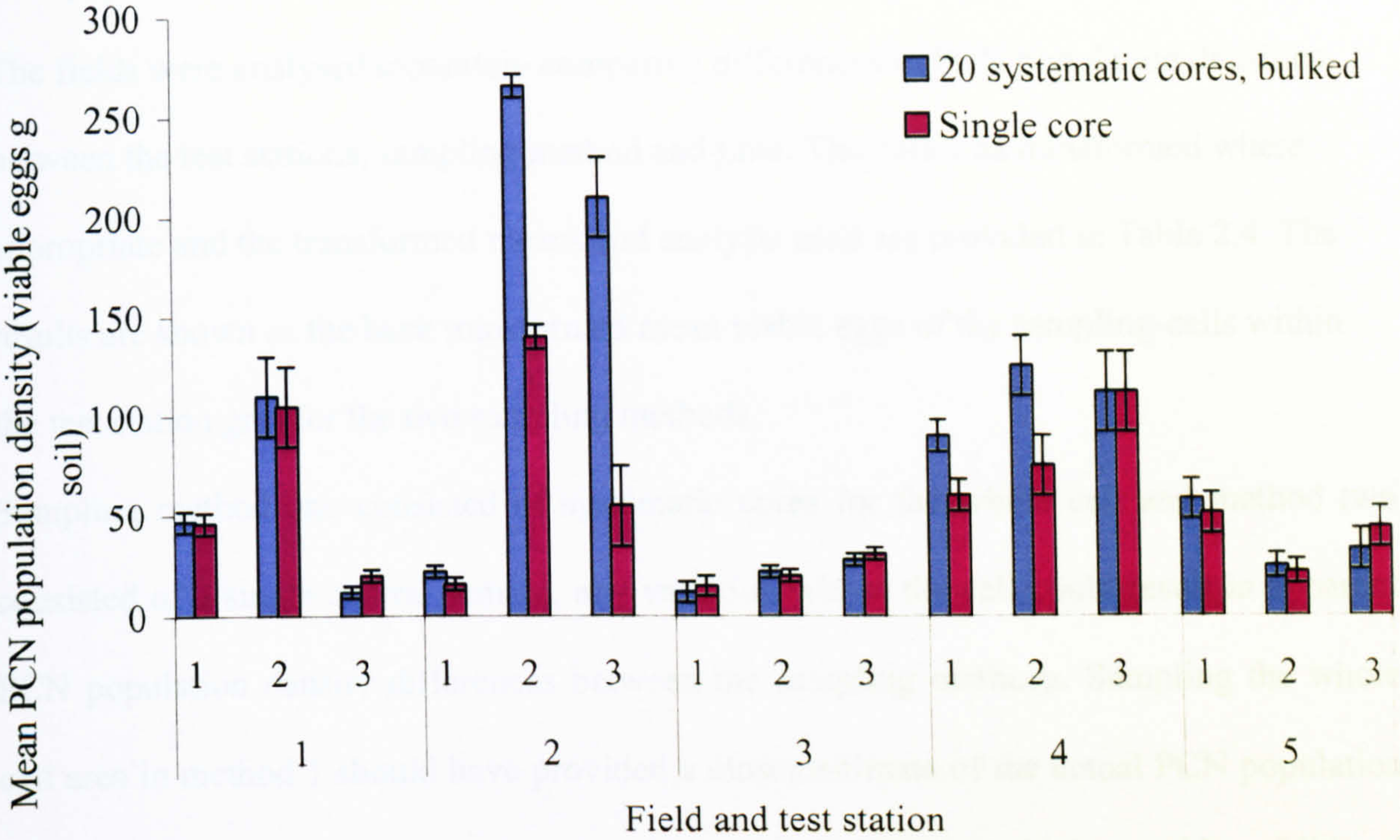


Figure 2.2 Mean PCN populations within the field test stations for 2000 (with +/- SE).

Table 2.3 Comparison between PCN counts for the mean of the 1 m^2 sub-divisions and the 2 m^2 total area.

		Number of PCN present (viable eggs g^{-1} soil)	
Field	Test station	Mean of 1 m^2 samples (SE)	2 m^2 sample
1	1	13.1 (3.7)	16.0
	2	32.9 (6.8)	39.9
	3	6.0 (2.3)	5.0
2	1	14.0 (4.2)	19
	2	20.7 (2.9)	19
	3	47.0 (15.6)	36.3
3	1	40.4 (8.4)	48.2
	2	147.3 (8.0)	163.6
	3	121.8 (36.2)	119.4
4	1	92.2 (18.6)	118.7
	2	107.9 (12.9)	136.2
	3	85.2 (4.9)	85.8
5	1	107.7 (11.6)	98.7
	2	49.54 (9.1)	46.9
	3	40.7 (5.4)	36.6
			p = 0.217

PCN population density analysis

The fields were analysed separately comparing differences in PCN population densities between the test stations, sampling method and time. The data was transformed where appropriate and the transformed means and analysis used are provided in Table 2.4. The results are shown as the back transformed mean viable eggs of the sampling-cells within the test station grid for the two sampling methods.

Sampling method one consisted of systematic cores for the whole cell and method two consisted of a single central sample, any variation within the cell could result in apparent PCN population density differences between the sampling methods. Sampling the whole cell area in method 1 should have provided a closer estimate of the actual PCN population present. However, the comparison between the two sampling methods provides additional confirmation of PCN population density variation within the test stations.

Table 2.4 PCN population data and analysis for test stations.

Field	Station	Sampling type *	Back transformed mean of viable PCN eggs per g soil and S.E. 2000 Ψ Mean	S.E.		2001 Ψ Mean	S.E.		2002 Ψ Mean	S.E.		2003 Ψ Mean	S.E.		GLM analysis and results (Significance)
1	1	1	46.8	7.7	(1.67)	22.7	2.7	(1.36)	17.3	1.8	(1.24)	17.3	2.7	(1.24)	
		2	45.3	8.1	(1.66)	37.8	8.6	(1.58)	16.2	3.6	(1.21)	20.5	4.7	(1.31)	Log 10 transformed Station* Sample* Year **p= 0.020**
	2	1	112.2	14.9	(2.05)	43.1	8.4	(1.63)	27.3	4.2	(1.44)	29.5	4.5	(1.47)	
		2	104.3	24.8	(2.02)	35.9	7.5	(1.56)	27.9	5.1	(1.45)	25.8	3.6	(1.41)	
	3	1	11.9	1.4	(1.07)	1.3	0.4	(0.12)	4.3	1.0	(0.63)	3.1	1.2	(0.49)	
		2	26.4	2.9	(1.42)	2.7	0.6	(0.43)	2.9	0.8	(0.46)	1.8	0.7	(0.27)	
2	1	1	29.6	1.9	(5.44)	16.9	4.9	(4.11)	16.9	2.6	(4.11)	45.4	4.9	(6.74)	
		2	20.6	2.8	(4.54)	15.1	2.8	(3.88)	12.1	1.8	(3.47)	33.6	5.3	(5.79)	Square root transformed Station* Sample* Year **p= 0.020**
	2	1	213.7	24.2	(14.62)	83.9	6.7	(9.16)	56.0	5.1	(7.49)	42.2	3.4	(6.12)	
		2	52.9	9.7	(7.27)	36.0	5.0	(6.00)	58.5	7.8	(7.65)	33.5	4.0	(5.78)	
	3	1	261.1	4.0	(16.16)	84.2	10.4	(9.18)	65.8	6.9	(8.11)	54.4	15.9	(7.37)	
		2	150.7	30.7	(12.27)	77.8	8.4	(8.82)	58.2	10.0	(7.63)	23.2	4.6	(6.79)	

Continued

3	1	1	100.9	20.0	(9.45)	74.0	9.1	(7.87)	62.8	10.2	(7.47)	35.7	6.5	(5.47)	Square root transformed Station* Sample* Year p= 0.634 Square root transformed Station* Year **p= 0.034**
		2	37.9	7.7	(7.04)	41.2	10.2	(7.29)	109.8	37.3	(10.81)	34.9	10.4	(6.33)	
	2	1	128.2	24.6	(11.32)	142.3	37.4	(11.93)	81.4	11.0	(9.02)	139.5	38.2	(11.81)	
		2	75.2	12.6	(8.67)	181.4	40.6	(13.47)	128.8	22.7	(11.35)	93.2	20.2	(9.65)	
	3	1	104.3	18.5	(10.21)	98.2	15.3	(9.91)	58.9	6.7	(7.67)	60.3	16.7	(7.77)	
		2	104.1	18.2	(10.20)	159.7	40.9	(12.64)	155.4	21.5	(12.47)	113.0	23.3	(10.63)	
4	1	1	10.2	2.0	(3.19)	45.5	8.0	(6.75)	67.7	12.1	(8.23)	23.1	3.1	(4.81)	Square root transformed Station* Sample* Year p= 0.640 Square root transformed Station* Year **p= 0.021**
		2	16.3	4.2	(4.04)	54.2	8.9	(7.36)	65.6	12.6	(8.10)	29.0	3.2	(5.39)	
	2	1	25.1	7.9	(5.01)	78.7	8.4	(8.87)	106.1	8.2	(10.30)	84.1	11.2	(9.17)	
		2	21.6	3.6	(4.64)	111.6	18.9	(10.56)	108.2	13.3	(10.40)	82.0	13.4	(9.05)	
	3	1	28.0	4.7	(5.29)	70.4	8.5	(8.39)	111.3	12.9	(10.55)	57.1	8.7	(7.55)	
		2	29.4	4.1	(5.42)	57.9	15.2	(7.61)	76.2	9.7	(8.73)	55.6	8.9	(7.46)	
5	1	1	53.0	14.8	(1.72)	97.7	10.5	(1.99)	101.5	16.8	(2.01)	66.1	8.0	(1.82)	Log 10 transformed Station* Sample* Year p= 0.811 Log 10 transformed Station* Year p= 0.348
		2	42.6	18.1	(1.63)	102.4	19.4	(2.01)	93.0	8.6	(1.97)	55.8	11.8	(1.80)	
	2	1	21.3	6.30	(1.33)	62.7	18.9	(1.80)	31.7	3.8	(1.50)	60.3	2.5	(1.32)	
		2	20.4	7.4	(1.31)	60.0	10.9	(1.78)	58.7	14.6	(1.77)	60.3	4.2	(1.37)	
	3	1	33.0	7.6	(1.52)	110.1	12.6	(2.04)	92.9	13.4	(1.97)	56.8	14.8	(1.96)	
		2	46.0	14.2	(1.66)	106.2	16.6	(2.03)	82.2	13.1	(1.91)	63.7	10.8	(1.88)	

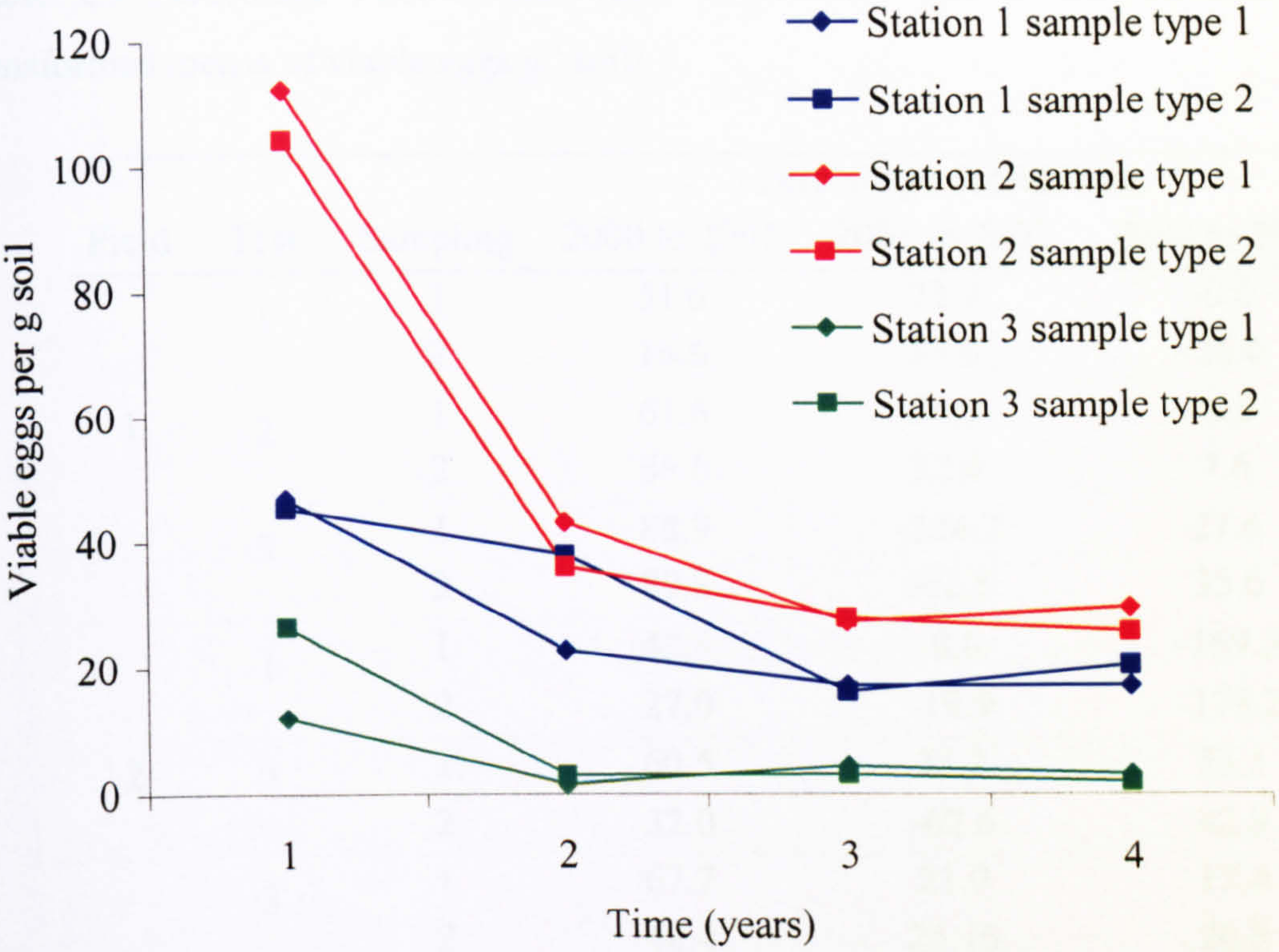
* = sample type (1= 20 systematic cores 2= single core)

Ψ = transformations in parenthesis

Fields 1 and 2

The results for the mean PCN populations in the two fields with pure *G. pallida* populations (with a potato crop in 1999) are shown in Figure 2.2. The decline rates are shown in Table 2.3. The population densities in the test stations of fields 1 and 2 were found to significantly differ over time, P= 0.020 and 0.023 respectively. In field 1 the decline rates between 2000 and 2001, post potato crop ranged from 16.6 to 88.9 %. Field 2 declines were less broad, ranging from 27 to 67.7 % declines. The range of decline observed here is far greater than that observed in other studies (Table 1.1). Although the graphs in Figure 2.3 do show that the declines in the first year were generally greater than those in the following years as found by den Ouden (1960).

The results of the 2001 to 2002 and 2002 to 2003 declines show increased variation in some test stations with some PCN population densities increasing i.e. Field 1, Station 3, for both sampling methods 1 and 2 (Table 2.5). It is not possible for PCN population densities to increase in the absence of a host crop. Volunteer potatoes from previous crops have been found to result in increases in PCN during non host crops in a rotation (den Ouden, 1967). However, the test stations and surrounding areas were monitored for volunteers and if present were removed. Therefore, a factor not accounted for using the test station design must have been responsible. This is most likely to be the mass movement of soil by cultivation. During seedbed preparation a number of cultivations are carried out in conventional systems prior to planting (see section 3.3). Cultivation operations have the potential to move soil and with it cysts in a number of directions and to varying distances (see section 5.6).



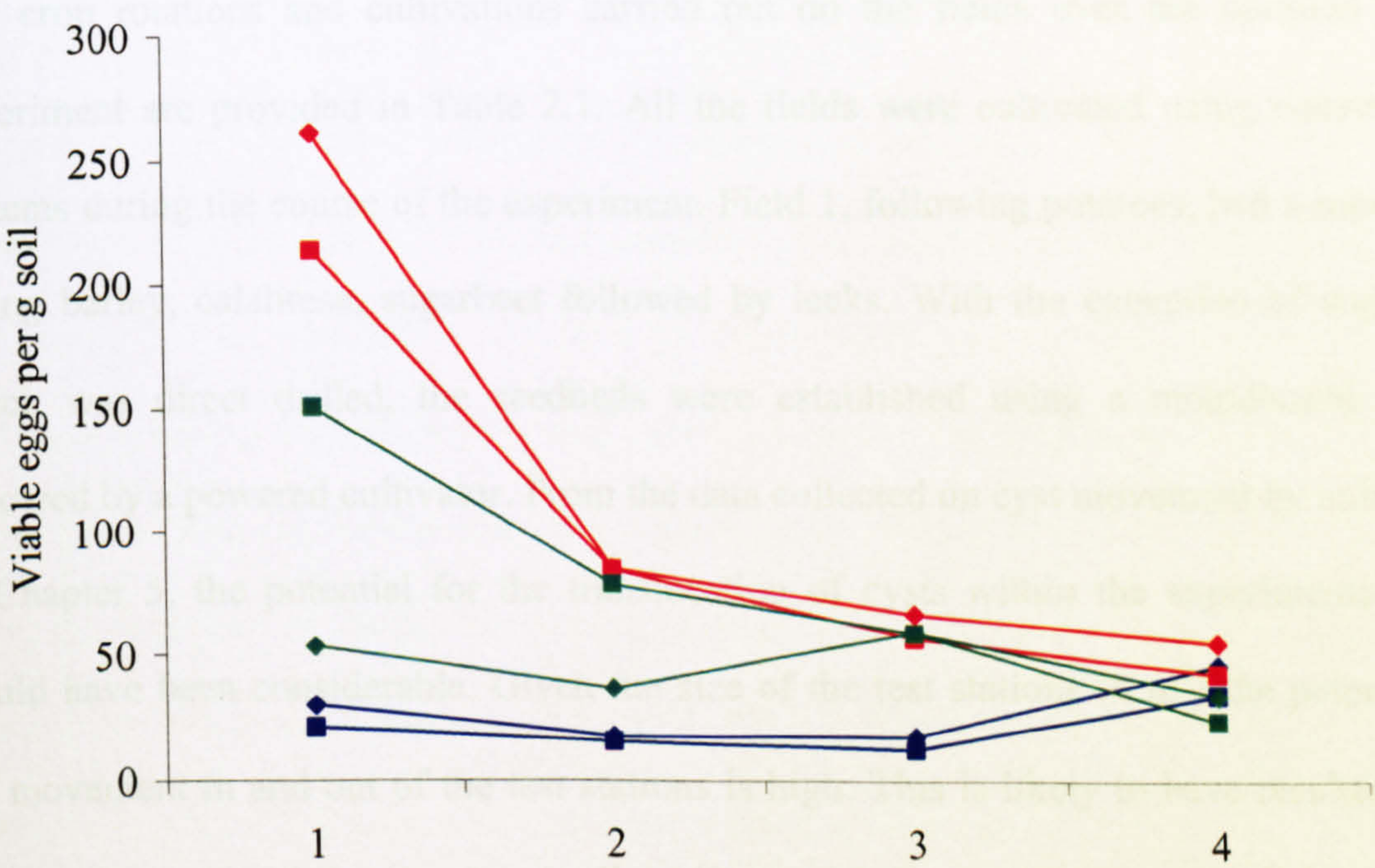


Figure 2.3 Back transformed means for PCN population densities within the test stations over time for Field 1 and 2, using two different sampling techniques (1= 20 systematic core, bulked, 2= 1 central core). * = potatoes crop year before first sample collected

Table 2.5 Percentage PCN decline rates in Fields 1 and 2 (derived from the back transformed means of viable eggs g^{-1} soil).

Field	Test	Sampling	Percentage decline rate 2000 to 2001	2001 to 2002	2002 to 2003
1	1	1	51.6	23.9	-0.0
		2	16.6	57.0	-26.0
	2	1	61.6	36.7	-8.2
		2	65.6	22.4	7.6
	3	1	88.9	-224.2	27.6
		2	89.8	-62.5	35.6
2	1	1	42.8	-0.0	-169.3
		2	27.0	-19.9	-178.2
	2	1	60.5	33.2	33.1
		2	32.0	-62.6	42.8
	3	1	67.7	21.9	17.4
		2	48.4	25.15	20.8

The crop rotations and cultivations carried out on the fields over the duration of the experiment are provided in Table 2.1. All the fields were cultivated using conventional systems during the course of the experiment. Field 1, following potatoes, had a rotation of spring barley, calabrese, sugarbeet followed by leeks. With the exception of sugarbeet, which was direct drilled, the seedbeds were established using a mouldboard plough followed by a powered cultivator. From the data collected on cyst movement by cultivation in Chapter 5, the potential for the translocation of cysts within the experimental fields would have been considerable. Given the size of the test stations (6 m^2) the potential for net movement in and out of the test stations is high. This is likely to have resulted in the soil that was sampled at each sampling date not being representative of the previous sample. This was also found by Wharton (1986), where population densities increases within field decline studies occurred during the spring and early summer. A possible suggested reason for this was the collection of a higher proportion of fuller cysts being sampled following redistribution by cultivation practices. However, this was dismissed due to what were perceived as only minimal cultivation practices at this time of year.

Fields 3,4 and 5

The PCN population dynamics for the three fields which had a potato crop during the first year of the experiment are shown in Figure 2.4 and the PCN decline rates in Table 2.6. There was a significant difference between the years for fields 3 and 4, p= 0.034 and 0.021 respectively (Table 2.4). No significant difference was found in the PCN population densities over time in Field 5 (p= 0.348). Over the first year of the experiment the PCN population densities within the tests stations generally increased. This was to be expected as the presence of a host crop meant that the PCN could complete their life-cycle and multiply. However, within two of the test stations in field 4, declines were found using sampling method 1. The results for the three fields overall failed to show consistent declines after potatoes. In field 3 PCN population densities increased for the year following potatoes. Overall the fields 3, 4 and 5 showed greater variation in PCN population densities than were found in fields 1 and 2 .

The results to confirm that cultivation practices were having a major effect on the PCN population densities within the test stations. The reason for the more pronounced differences in the population densities of these fields may be due to the cultivation practices involved before, during and after a potato crop (Table 2.1). The cultivation operations carried out for a potato crop result in large amount of soil movement. The fields were sampled prior to the potatoes being planted. This meant prior to the formation of the potato beds which are then de-stoned and bed tilled. These individual operations have the potential to move a cyst over 3 m both with the direction of cultivation, behind and laterally (see section 5.6.3). The fields were then planted resulting in PCN multiplication within the bed, and therefore PCN aggregation around the potato plants. Subsequently the potatoes are harvested and the beds have to be flattened before the next crop resulting in more soil translocation.

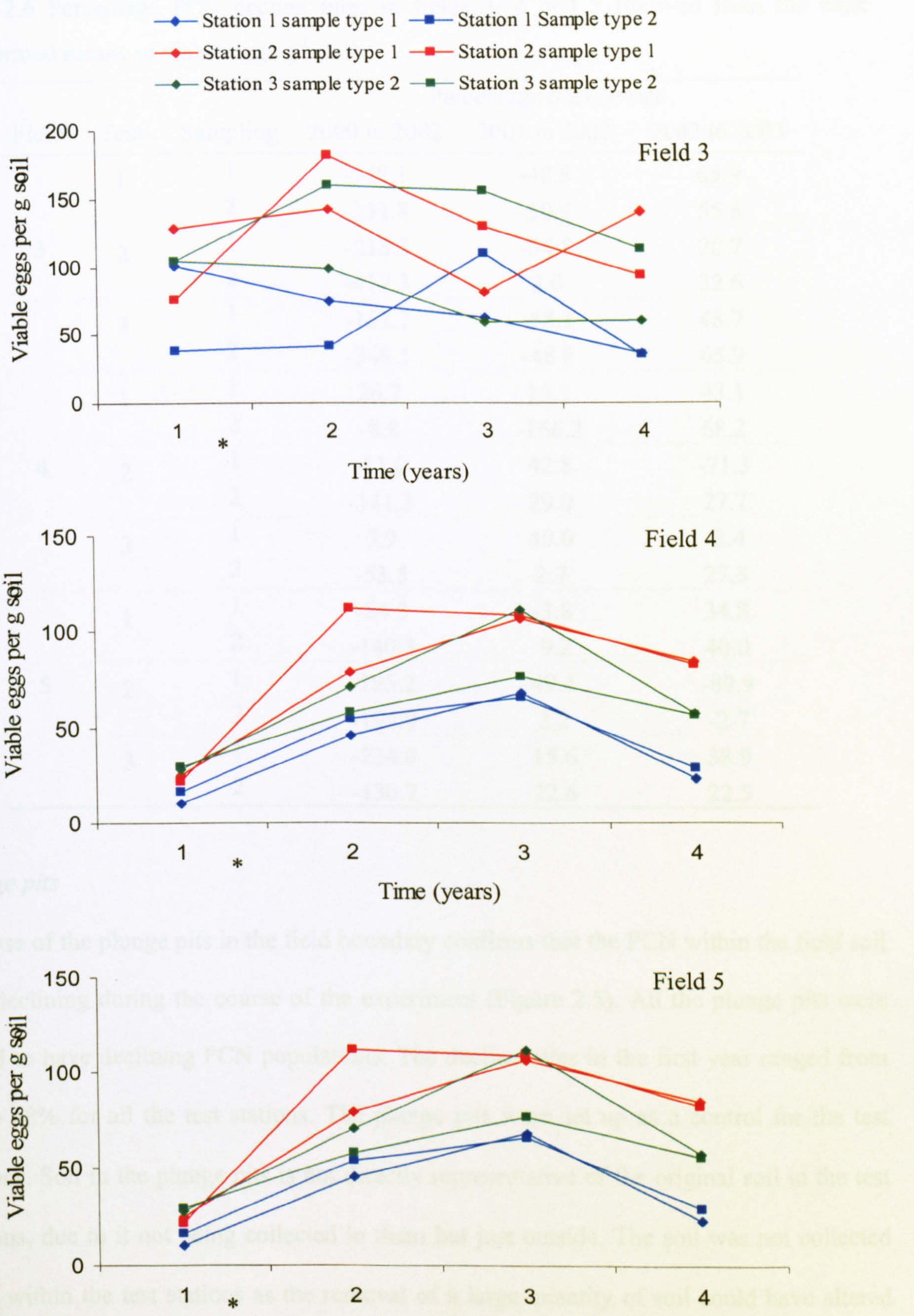


Figure 2.4 Back transformed means for PCN population densitiess within the test stations over time for Fields 3, 4 and 5, using two different sampling techniques (1 = 20 systematic core, bulked; 2 = 1 central core). * = Potato crop grown

Table 2.6 Percentage PCN decline rates in fields 3, 4 and 5 (derived from the back transformed means of viable eggs g^{-1} soil).

Field	Test	Sampling	Percentage decline rate 2000 to 2002	2001 to 2002	2002 to 2003
3	1	1	-348.1	-48.8	65.9
		2	-231.8	-20.9	55.8
	2	1	-213.3	-34.8	20.7
		2	-417.3	3.0	32.6
	3	1	-151.7	-58.1	48.7
		2	-348.1	-48.8	65.9
4	1	1	26.7	15.1	43.1
		2	-8.8	-166.2	68.2
	2	1	-11.0	42.8	-71.3
		2	-141.3	29.0	27.7
	3	1	5.9	40.0	-2.4
		2	-53.5	2.7	27.3
5	1	1	-84.3	-3.8	34.8
		2	-140.3	9.2	40.0
	2	1	-195.2	49.4	-89.9
		2	-194.0	2.2	-2.7
	3	1	-234.0	15.6	38.9
		2	-130.7	22.6	22.5

Plunge pits

The use of the plunge pits in the field boundary confirms that the PCN within the field soil was declining during the course of the experiment (Figure 2.5). All the plunge pits were found to have declining PCN populations. The decline rates in the first year ranged from 12 to 68% for all the test stations. The plunge pits were set up as a control for the test stations. Soil in the plunge pits is not directly representative of the original soil in the test stations, due to it not being collected in them but just outside. The soil was not collected from within the test stations as the removal of a large quantity of soil could have altered the PCN population density. The plunge pits provide information on what would have resulted in the test stations had the soil sampled remained constant. No direct comparison between the plunge pits is possible as no replicate samples were taken and fields 3, 4 and 5

had different durations since the last potato crop. Additionally six plunge pits were destroyed in the field boundaries by cultivation during the course of the experiment.

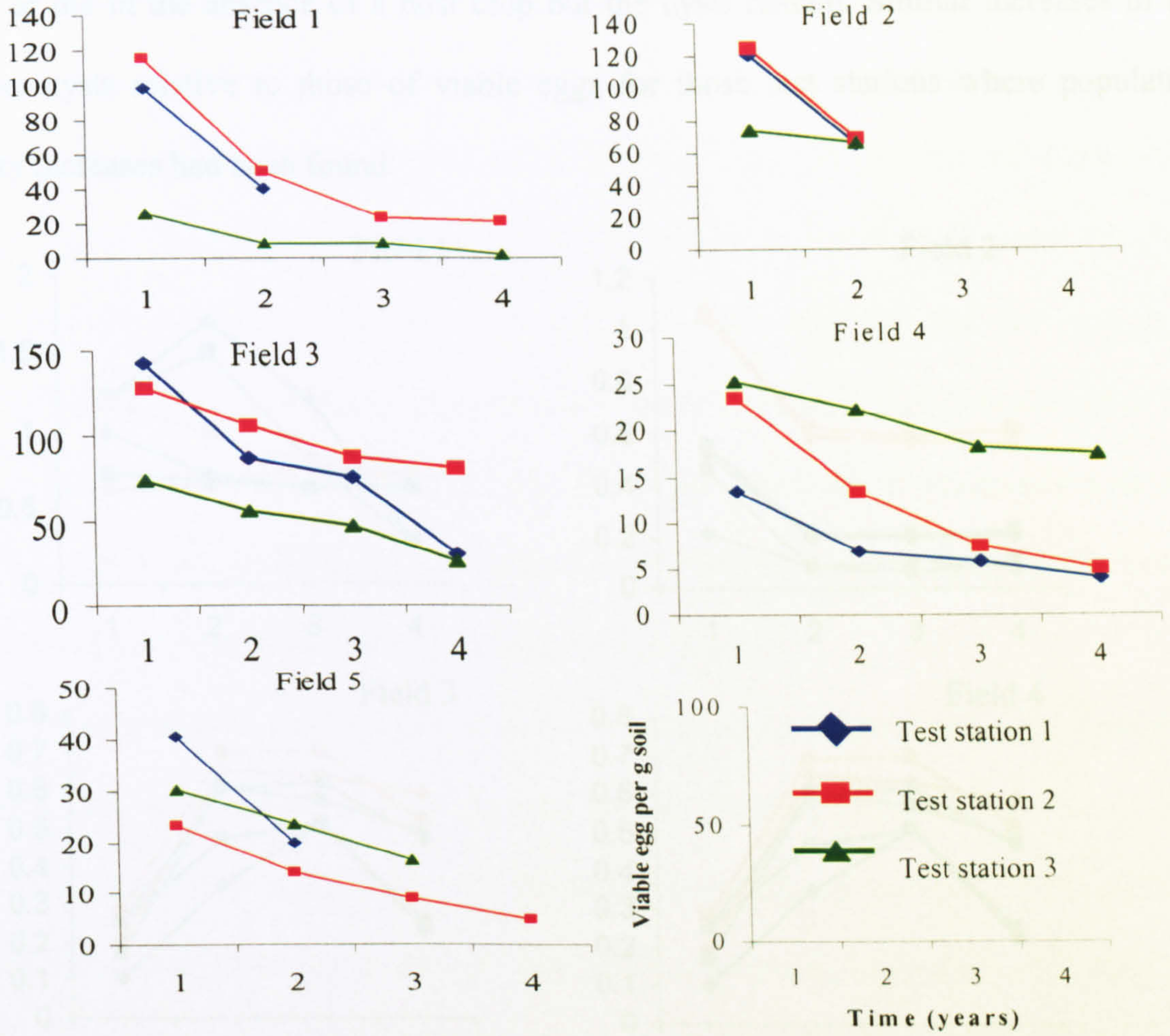


Figure 2.5 PCN population densities within plunge pits for the five experimental sites.

Viable egg/cyst ratio

If the cultivation operations were responsible for the alteration of population densities in the test stations this would be represented by changes in the number of cysts (Figure 2.6). A further statistical analysis was carried out to determine the correlation between the number of viable eggs g^{-1} soil and cysts g^{-1} soil using Pearsons Correlation. This was carried out using the full data set, not mean values. Correlation of 0.762 was found indicating a strong correlation. This suggested a strong correlation between the cyst and eggs numbers. This may be expected initially after a potato crop as new cysts contain a

relatively constant number of eggs. Over time as the population density declines the cysts should not necessarily decline relative to the number of eggs i.e. a percentage of the eggs hatch or die in the absence of a host crop but the cysts remain. Similar increases in the number cysts relative to those of viable eggs for those test stations where population density increases had been found.

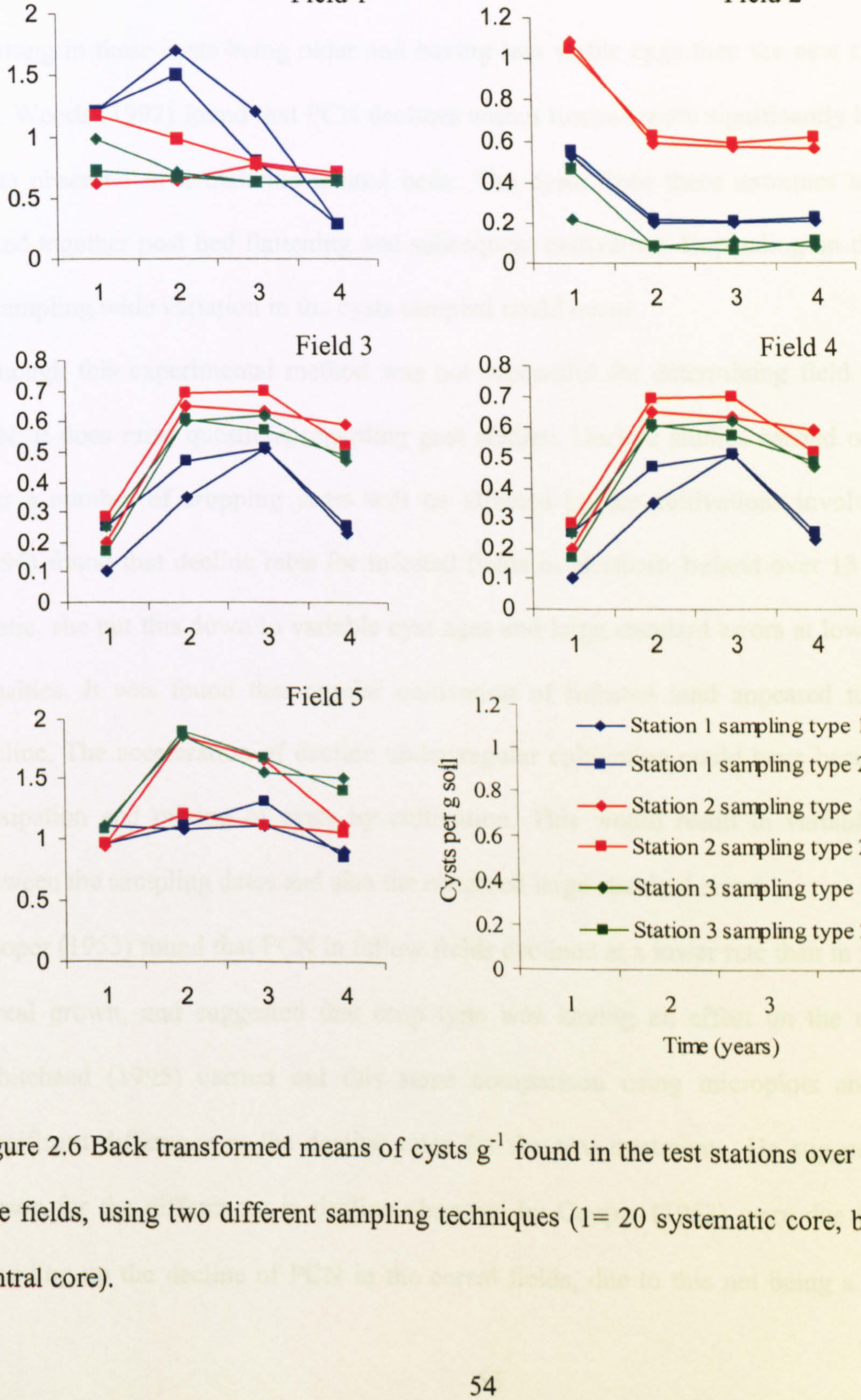


Figure 2.6 Back transformed means of cysts g^{-1} found in the test stations over time for the five fields, using two different sampling techniques (1= 20 systematic core, bulked, 2= 1 central core).

The decline rate could be monitored by comparisons between the viable eggs per cyst. However this would require the assumption that the cysts being moved to a location had a matching history to those moved away. This assumption is not acceptable due to the nature of potato production. Potatoes are planted in beds, where new PCN are be produced on the potato roots. Whereas juveniles of cysts in the furrows are unlikely to reach the roots, resulting in these cysts being older and having less viable eggs than the new cysts in the bed. Woods (1997) found that PCN declines within furrows were significantly higher than those observed in nematicide treated beds. The cysts from these extremes are then be mixed together post bed flattening and subsequent cultivation. Depending on the location of sampling wide variation in the cysts sampled could occur.

Although this experimental method was not successful for determining field declines of PCN, it does raise questions regarding past studies. Decline studies carried out in fields over a number of cropping years will be affected by the cultivations involved. Turner (1996) found that decline rates for infested fields in Northern Ireland over 13 years were erratic, she put this down to variable cyst ages and large standard errors at low population densities. It was found that regular cultivation of infested land appeared to accelerate decline. The acceleration of decline under regular cultivation could have been due to the dissipation and mixing of cysts by cultivation. This would result in variable cyst ages between the sampling dates and also the observed large standard errors.

Cooper (1953) found that PCN in fallow fields declined at a lower rate than in fields with a cereal grown, and suggested that crop type was having an effect on the decline rate. Whitehead (1995) carried out this same comparison using microplots and found no significant difference in the decline rates for the two treatments. He suggested that the reason for the differences in decline observed by Cooper (1953) were due to ploughing speeding up the decline of PCN in the cereal fields, due to this not being a factor in his

microplot design. The reason for the observed decline rate difference is more likely to have been due to the horizontal movement of cysts by cultivation. There have been no studies carried out investigating the direct effect of cultivation on the physical damage or decline of PCN. In India it has been found that multiple summer ploughings reduces population densities of the cereal cyst nematodes (*Heterodera avenae*) (Mathur *et al.*, 1991). However, they attribute the decline to the cysts being brought to the surface and exposed to solar heat. This is unlikely to be a major contributing factor when single ploughing in the UK. In addition to this the increased yields of cereal following this summer ploughing may be due to the dispersal of cysts by multiple ploughing as well as their destruction by solar heat i.e. if the cysts are widely dispersed then invasion of individual plants may not reach a damage threshold.

From this experiment the use of point samples or sample areas may give estimates of population densities for a location but this is likely to alter temporally following cultivation operations. Soil removed from the field and placed in plunge pits could be a more accurate method for monitoring PCN decline rates. This method is employed in Experiment 2.

2.4 Experiment 2. Natural decline of PCN in the absence of a host crop, using field soil in plunge pits

2.4.1 Introduction

The aims of this experiment were to investigate the extent of variation within the natural decline of PCN within commercial potato fields in England and to establish whether any variation in decline could be attributed to specific factors such as species and soil type. The experiment consisted of establishing plunge pits with PCN-infested soil to remove any potential effects of cultivation practices on the natural decline of the PCN population densities. This experiment was set up in March 2002.

2.4.2 Material and Methods

2.4.2.1 Field selection

The maximum number of PCN populations that it was considered could be set up and monitored was fifty and this number was used as the basis for the experimental design. The fifty sites were allocated to the counties proportionally to the hectarage of potatoes grown (Table 2.7) to provide a national perspective for commercial potato fields.

The fields were selected from information provided by Agrovista agronomists. The criteria for selection were, that they were infested with PCN and had grown a potato crop in 2001. Following consultation with the agronomists 45 sites that met the criteria were found. From each of these fields around 30 kg of topsoil was collected. The location in the fields for the collection of soil was determined using PCN distribution field maps and information provided by the growers. The field information for the location of the sampled soils is provided in Table 2.8.

Table 2.7 Determination of field site locations in England and Wales , by area (hectares) of potatoes grown. (Anon., 2000)

County	Hectares	As % of England/Wales	For 50 sites
Lancashire	5690	5.0%	3
Lincolnshire	16039	14.2%	7
Norfolk	13094	11.6%	6
Kent	2499	2.2%	1
Leicestershire	803	0.7%	0
Northamptonshire	206	0.2%	0
Essex	3634	3.2%	3
Gloucestershire	818	0.7%	0
Hertfordshire	247	0.2%	0
Durham	753	0.7%	0
Hampshire	974	0.9%	1
Herefordshire	3005	2.7%	1
Cheshire	5243	4.7%	2
Cornwall	5023	4.5%	2
Dorset	188	0.2%	0
Cumbria	870	0.8%	0
Derbyshire	781	0.7%	0
Devon	1923	1.7%	1
Berkshire	98	0.1%	0
Bucks South	95	0.1%	0
Cardigan	202	0.2%	0
Bedfordshire	796	0.7%	0
Brecon	204	0.2%	0
Cambridgeshire	10414	9.2%	5
Suffolk	5459	4.8%	2
Somerset	2056	1.8%	1
Yorkshire	14173	12.6%	6
Warwick	1611	1.4%	1
Surrey	48	0.0%	0
Sussex West	880	0.8%	1
Wiltshire	490	0.4%	0
Worcester	925	0.8%	1
Sussex East	291	0.3%	0
Northumberland	545	0.5%	0
Oxfordshire	310	0.3%	0
Nottinghamshire	3076	2.7%	1
Shropshire	5792	5.1%	3
Staffordshire	3442	3.1%	2
Total	112697	100.0%	50

Table 2.8 Plunge pit field soil information.

County	Soil type	Species present	Initial PCNPopulation (viable eggs g^{-1} soil)
Cambridgeshire	Clay loam	mix	36.8
Cambridgeshire	Peat	*G.rostochiensis*	8.9
Cambridgeshire	Sandy silt loam	*G. pallida*	130.4
Cambridgeshire	Peat	mix	171.9
Cambridgeshire	Peat	*G.rostochiensis*	346.7
Cornwall	Sandy silt loam	*G. pallida*	3.9
Cornwall	Sandy loam	mix	6.4
Devon	Clay loam	*G. pallida*	13
Kent	Clay loam	*G.rostochiensis*	119.2
Kent	Clay loam	*G.rostochiensis*	37.3
Lancashire	Peat	*G.rostochiensis*	6.3
Lancashire	Peat	*G. pallida*	156
Lancashire	Peat	*G. pallida*	25.3
Lancashire	Peat	*G. pallida*	20.9
Lincolnshire	Sandy clay loam	*G. pallida*	49.6
Lincolnshire	Sandy clay loam	*G. pallida*	4.3
Lincolnshire	Sandy loam	*G. pallida*	48.3
Lincolnshire	Sandy loam	*G. pallida*	698.9
Lincolnshire	sandy clay loam	*G. pallida*	344.5
Norfolk	Sandy loam	mix	16.6
Norfolk	Sandy silt loam	*G.rostochiensis*	144.4
Norfolk	Sandy silt loam	*G. pallida*	20.8
Norfolk	Sandy silt loam	*G. pallida*	86.2
Nottinghamshire	Sandy loam	*G. pallida*	8.7
Shropshire	Sandy loam	mix	202.8
Shropshire	Sandy loam	*G. pallida*	9.8
Shropshire	Sandy loam	*G. pallida*	194.9
Shropshire	Sandy loam	*G. pallida*	267.3
Staffordshire	Peat	mix	7.2
Staffordshire	Sandy loam	mix	96.4
Staffordshire	Sandy loam	*G.rostochiensis*	20.2
W Sussex	Clay loam	*G.rostochiensis*	5
Warwickshire	Sandy loam	*G.rostochiensis*	24.5
Warwickshire	Clay loam	*G. pallida*	51.1
Warwickshire	Sandy loam	*G. pallida*	13.1
Yorkshire	Sandy loam	*G. pallida*	12.5
Yorkshire	Sandy loam	*G. pallida*	6.7
Yorkshire	Sandy loam	*G. pallida*	51.1
Yorkshire	Sandy loam	mix	5.5

2.4.2.2 Experimental design

On a site at Harper Adams University College, 45 plunge pits were constructed, by submerging plastic pots (50 cm diameter by 50 cm depth, with drainage holes in the base) in the ground (Plate 2.1). The soil collected from the 45 fields were spread thinly on a concrete surface and rolled with a concrete roller to reveal any volunteer potatoes; any found were removed. The soil was then thoroughly mixed, in a cement mixer, and then placed in a plunge pit.

Plate 2.1 Plunge pit location at Harper Adams University College (Autumn, 2004).

2.4.2.3 Plunge pit sampling

The pits were sampled at four month intervals, using a cheese type corer (5 cm diameter by 30 cm depth). Bulked sample of three cores; this was repeated three times to produce three replicate samples per pit. Before sampling the soil was removed from the pits and mixed in a cement mixer. The mixer was thoroughly cleaned between soils to ensure no cross contamination. The pits were monitored regularly to ensure they remained clear of weeds, these were removed by hand weeding. The samples were processed by standard methods described in section 2.2.

2.4.2.4 Data analysis

The data was analysed for number of viable eggs g^{-1} soil; this was converted to proportions of the initial population densities for statistical comparison. The proportional data for viable eggs g^{-1} soil was found to have a normal distribution. Data were analysed using GLMs for multiple analysis of variance; Tukey tests were performed where significant differences were found between population groupings. All analysis was carried out using Mintab 12 (MINITAB INC.).

2.4.3 Results and discussion

The results are discussed by overall variations between the populations and also for the factors of species present and soil type. Temporal variations are shown and discussed in terms of the first and second growing seasons following the potato crop. The reason for this is due to the experiment being conducted over an eighteen-month period. Therefore, only one complete annual decline rate for the PCN populations was obtained.

Following initial sample processing six plunge pits were found to contain soil with no or too low population densities of PCN for purposes of this experiment. These pots were subsequently not used in this study. The remaining 39 soils were composed of 22 *G. pallida*, 9 *G. rostochiensis* and 8 mixed populations. The soil types in the experiment consisted of 17 sandy loams, 6 clay loams, 8 peats, 5 sandy silt loams and 3 sandy clay loams (Table 2.8).

Initial analysis was carried out to determine if there were significant differences between all the populations over time. Significant differences occurred between the declines of the population densities over time (P= 0.024) (Figure 2.7). The population declines were found to range from 11 to 69% for the first year. However, there was no significant difference observed in the size of the declines between the sampling points. Although the decline rate

over the first growing season was higher in relation to the second growing season decline rate this was not significant, 27.1% (+/- 3.2) and 24.7% (+/- 3.7) respectively. This appears to contradict den Ouden (1960) who found that the initial year decline was higher than in the subsequent years. Their work was undertaken before the sub-division of PCN into two species. Additionally their study was run over three years in the field; the observed higher decline rate in the first year compared with the subsequent years could have been due to changes in population densities by cultivation practices. The potential effect of cultivation on PCN population dynamics was not present in this plunge pit experiment. The higher decline of PCN in the first year may also be attributable to differences within the species;, this could be masked by looking at the decline rate over time for all the plunge pits. Devine *et al.* (1999) found a decrease in decline in the second year post potatoes for *G. rostochiensis*. However, this work consisted of different fields being monitored between the decline years. The direct comparison between field populations between years may have been responsible for the observed differences.

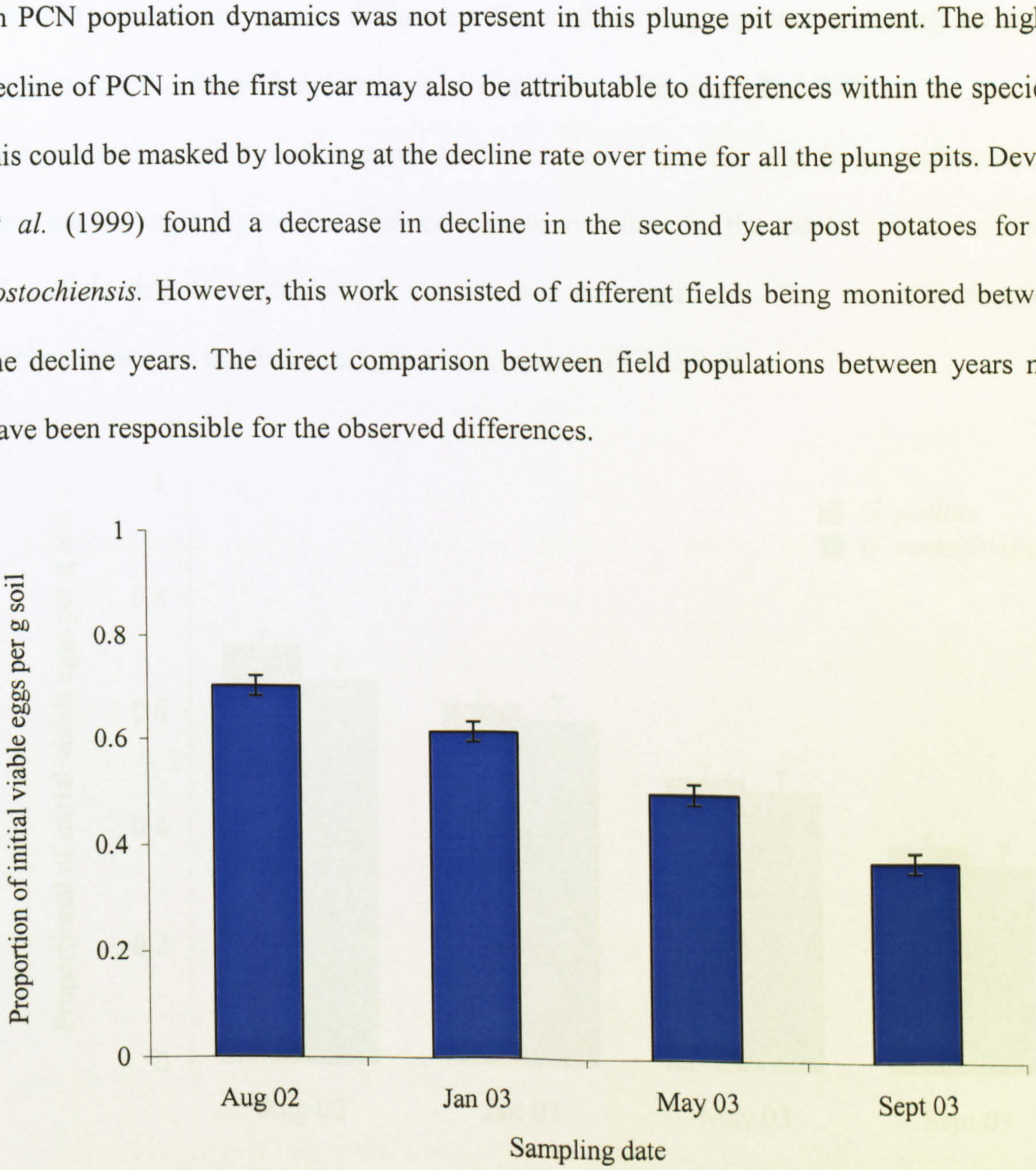


Figure 2.7 Proportional decline of the mean for all PCN populations over time (with +/- SE bars).

Species The effect of species was analysed over time for all the pots grouping the 8 mixed populations as a separate level of species. This found no significant difference between species over time (P= 0.972). The grouping of the mixed populations could have resulted in possible errors in the analysis due to all the mixed populations showing different ratios between *G. pallida* and *G. rostochiensis* present. To ensure the mixed population were not distorting any actual species differences the analysis was repeated removing the mixed populations; this found that there was no significant difference (P=0.890).

The proportions of the initial populations between *G. pallida* and *G. rostochiensis* over time are shown in Figure 2.8. The decline rate over time for the species was lower for the *G. pallida* than *G. rostochiensis* but this was not significant. This was also found for decline rates over the first year between the species (Table 2.9).

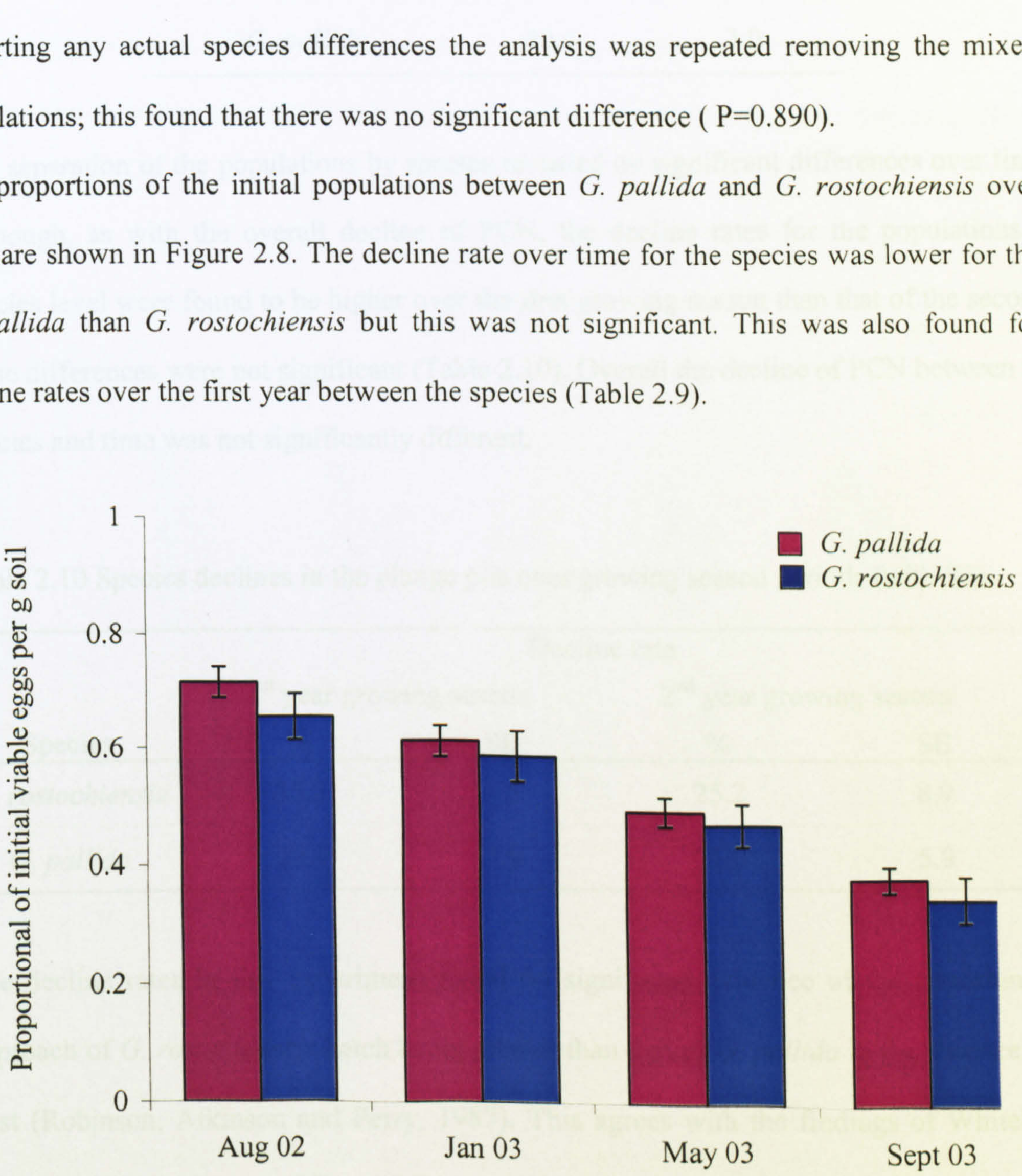


Figure 2.8 Mean proportional decline of *G. pallida* and *G. rostochiensis* in the plunge pits over the duration of the experiment (with +/- SE bars).

Table 2.9 Percentage population decline of *G. pallida* and *G. rostochiensis* in the plunge pits for the first year (with SE).

	Decline rate (1st year)	
Species	%	SE
G. rostochiensis	42.0	6.6
G. pallida	40.4	3.0

The separation of the populations by species revealed no significant differences over time. Although, as with the overall decline of PCN, the decline rates for the populations at species level were found to be higher over the first growing season than that of the second, these differences were not significant (Table 2.10). Overall the decline of PCN between the species and time was not significantly different.

Table 2.10 Species declines in the plunge pits over growing season periods (with SE).

	Decline rate			
	1st year growing season		2nd year growing season	
Species	%	SE	%	SE
G. rostochiensis	35.2	5.5	25.2	8.9
G. pallida	29.4	3.9	21.0	5.9

The decline rates in this experiment found no significant evidence of the opportunistic approach of *G. rostochiensis* hatch being greater than that of *G. pallida* in the absence of a host (Robinson, Atkinson and Perry, 1987). This agrees with the findings of Whitehead (1995), who found no significant difference between *G. pallida* and *G. rostochiensis* over time; this study was also conducted on infested soil removed from fields.

Soil type Due to species showing no significant difference, the analysis was carried out for the whole data set excluding species as a factor. This found that there was a significant difference between the PCN population densities in the different soil types (P = 0.024). The proportions of decline of the PCN populations in the different soil varied over time (Figure 2.9). It was found that the populations in the different soil type showed different levels of decline between the sampling dates. The sandy loam, clay loam and sandy clay loam soils were found to decline relatively consistently between the sampling points, whereas the peat and sandy silt loam showed more erratic declines between sampling points. The soils containing clay were found to have declined the lowest over the duration of the experiment.

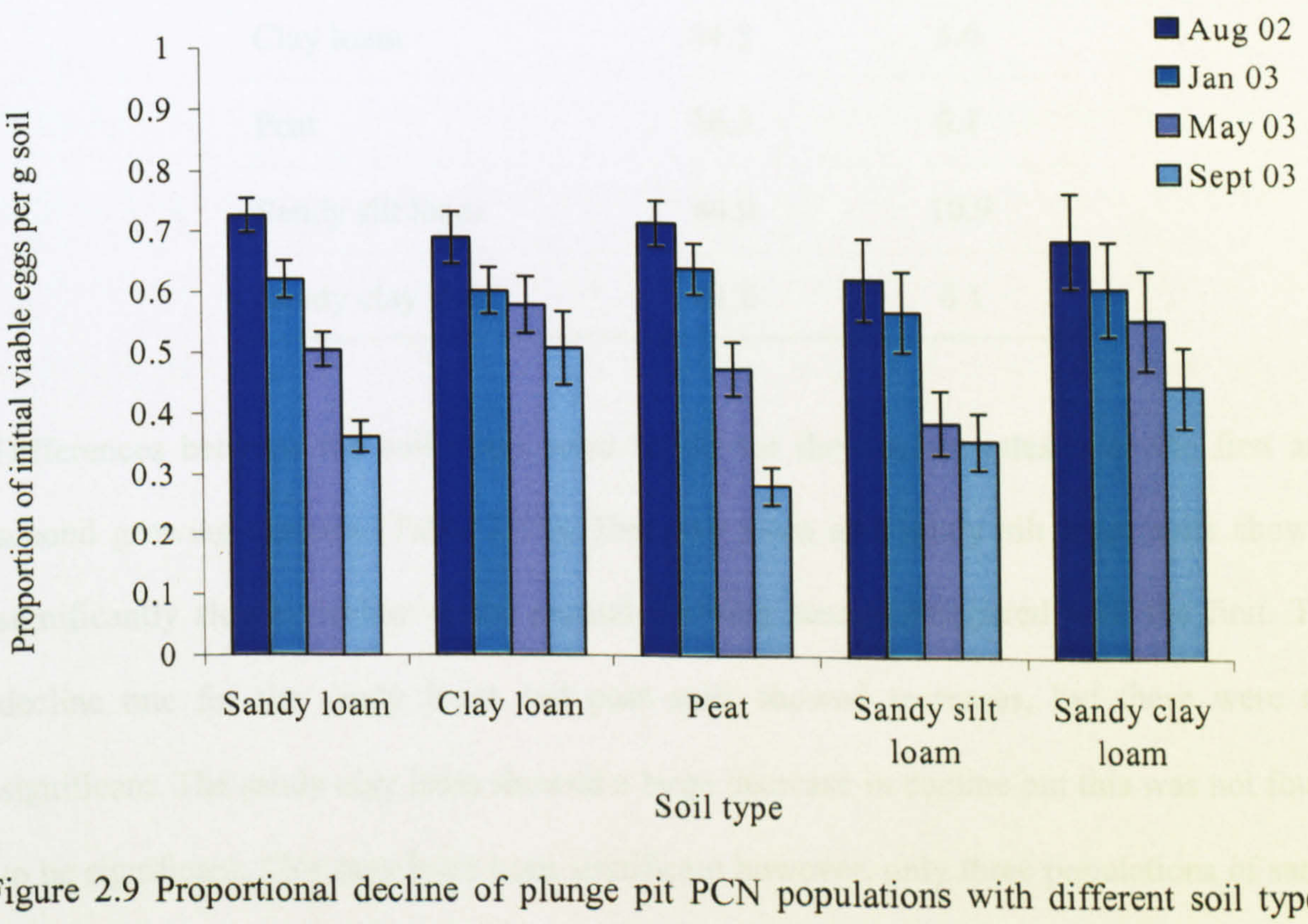


Figure 2.9 Proportional decline of plunge pit PCN populations with different soil types over time (with +/- SE bars).

The decline rate differences between the soil types were not found to be significantly different for the first year decline rate (Table 2.11). The PCN population densities in the different soil types were found to have declined by between 36.3 and 44.5%; the peat and

clay loam soils showed the lowest and highest declines, respectively. This agrees with the findings of Whitehead (1995), who found that the decline of PCN was not dependent on soil type, although this appears to contradict other studies that have found significant differences in PCN decline for different soil types (i.e. Grainger, 1960; Storey, 1984; Wharton, 1986 and Turner, 1996;).

Table 2.11 Percentage population density decline of the plunge pits with different soil types during the first year (with SE).

Soil type	Decline rate (1st year)	
	%	SE
Sandy loam	40.5	4.6
Clay loam	44.5	5.6
Peat	36.3	6.1
Sandy silt loam	44.0	10.9
Sandy clay loam	41.8	8.1

Differences between the soil types were found for the decline rates over the first and second growing seasons (Table 2.12). The clay loam and sandy silt loam soils showed significantly slower decline in the second growing season compared with the first. The decline rate for the sandy loam and peat soils showed increases, but these were not significant. The sandy clay loam showed a large decrease in decline but this was not found to be significant. This may have been significant however, only three populations of sandy clay loam soil were present in this study and they had highly variable declines. The three populations showed high levels of variation in decline, resulting in a high standard error for the mean of this soil type. Significant differences were found between the soil types during the second growing season. With the exception of the sandy clay loam soil, sandy

silt loam declined significantly less than all the other soil types. Peat and sandy loam declined greater than the other soil types for growing season two.

Table 2.12 Soil type and PCN declines over growing season periods (with SE).

Soil type	Decline rate			
	1st year growing season		2nd year growing season	
	%	SE	%	SE
sandy loam	28.4	3.7	30.7	5
clay loam	36.5	5.6	20	10.5
peat	28.8	5.6	32.5	6.8
sandy silt loam	39.3	10.8	8.5	4.6
sandy clay loam	30.2	14.7	16.1	10.2

Peat soils showed the highest PCN decline for the duration of the experiment. It has been suggested that the rotation length for peats and medium soils can be shorter than that for light silt soils (Anon., 1977). This contradicts Grainger (1960) who proposed that soils with high peat content would decline more slowly than sandy/loam soils, although, this may have been due to variation between the bulk densities of peat and lighter soils. This was not a contributing factor for this experiment due to the declines being determined by proportional changes, from the same soils using a standard sampling technique. Turner (1996) also found that PCN populations in peaty soils declined slower than for sandy soils, although this was not significant. A possible reason for the higher rate of decline for peat in this experiment could have been due to soil temperature changes. Peat as a dark soil has a greater potential to heat up by solar radiation than lighter soil. Increased soil temperature in the peat pits may have increased the rate of PCN hatch. Devine *et al.* (1999) found that rate of PCN hatch was increased at higher soil temperatures. The peat soils in this experiment were found to have higher decline rates during the growing season months compared with those over winter; this may have been as a result of temperature changes.

This was also suggested from the sandy loam soils, which were found to have declined overall similar to that of the peat; however, no similar trends in decline between summer and winter periods were found. Peat was the only soil type found to be showing seasonal fluctuations in the rate of decline. Overall the variation within the PCN declines within the soil types was high during the experiment; sandy loam 39 to 89 %, clay loam 27 to 85 %, peat 52 to 81 %, sandy silt loam 38 to 78 % and sandy clay loam 36 to 73 %. The decline rate changes between the soil types over the duration of the experiment seem to be in line with Wharton (1986) who found differences between high levels of variation within soil types; sandy loam 5 to 59 %, peat 10 to 54% and clay loam 31 to 59 %.

The observed variations were not accounted for by either species or overall by soil type in this work. This suggests that other factors within the field soil are having an effect on decline rates in commercial potato fields in England. These factors may include the presence of antagonistic micro-organisms, resulting in higher in-egg mortality (Whitehead, 1997). A major potential factor resulting in the variation between PCN population densities observed in this work and the previous studies is the composition of the PCN population. Decline rates have been found to be independent of population density but are age dependent (Whitehead *et al.*, 1980). This was suggested in experiment 1 for the variations found in the test station declines; that if the cysts are being moved by cultivation then different-aged cysts may have been sampled during the course of the experiment. Although the use of plunge pits in experiment 2 removed the factor of cultivation movement, the potential for different aged populations remained for comparisons between the different PCN populations. By sampling the fields post potatoes it was hoped that the declines would be for new populations however, this would still be dependent on the sampling location within a field. The age of the cysts sampled would have been dependent on

whether the location sampled was soil from within a bed or a furrow and the amount of mixing of these soils that had occurred post potatoes. If the soil had all been obtained from bed soil then the proportion of new cysts is likely to have been higher than if mixed with furrow soil. This could have resulted in the PCN populations being composed of varying ages of cysts. Potentially, this could have resulted in the observed high variations between the PCN populations within the species and soil groupings. Within the PCN populations this variation is likely to occur within a field, resulting in variations within the field overall decline rate.

The *Globodera* spp. present within a field is important for management strategies, such as the potential for limiting multiplication within a potato crop by growing resistant cultivars and the potential effects nematicide applications can be expected to have on the PCN population. However, this was not found to be a significant factor for the population density decline rates. This suggests that the species present should not directly affect the length of rotation. Indirectly, however, within an integrated management strategy it will be important for combining rotation length with other control measures.

The variations in decline rates for the thirty-nine populations have important implications for control measures. *Globodera* ssp. can be controlled by a number of control measures used in conjunction with each other, in which rotation plays an important role. Using the standard 30 % y^{-1} decline rate a population of 50 eggs g^{-1} soil would require a rotation length of 6.5 years between potato crops to return it to 5 eggs g^{-1} soil. However if the decline rate was 55 % y^{-1} the interval between potato crops would be less than 3 years but 22 years is required if the decline rate is 10 % y^{-1}. This clearly shows that the decline rate for a PCN population density will greatly influence the frequency of potato cropping, if only rotational control is used. It will also be of importance when using rotation as part of a integrated management strategy. Using a known decline rate for a field would result in

more robust information on expected population dynamics within a integrated management strategy computer simulation model (Trudgill *et. al*, 2003).

If the decline rates found in this study were maintained over several years in fields considerable differences would result within the populations. By looking at the minimum and maximum declines found in this study the potential variation in field population densities is high. If a field population declined at 69% y^{-1} and had an initial population density of 100 egg per g^{-1} soil after 3 years the population density would be under 3 eggs g^{-1} soil. In contrast, the PCN population density found to be declining at 11 % y^{-1} would have a population density of 82 eggs g^{-1} after 3 years at the same initial population density. This shows that the minimum duration of rotation required to maintain potato production on fields is not consistent.

The variation in decline rates of field populations in this experiment confirms those found in earlier studies, with declines ranging from 10 to 60% per year. The implications for this work is that using a standard decline rate of 30 % may result in longer or shorter rotations than are required to continue commercial potato production on a field. This experiment also seems to confirm that cultivations were having a significant effect on the populations sampled in experiment 1. This suggests that the use of populations removed from an infested field is the best way to monitor the decline of PCN field populations, although within-field sampling should also be implemented prior to a potato crop. The use of both techniques would provide information on the population within the field and additionally the decline rate that can be expected from that population. The setting up of a plunge pit post potato crop would allow the collection of several years decline data before the next potato crop in the rotation. If several plunge pits were setup with soil from different locations the potential for variation in age of cysts in the pits relative to the field populations could be reduced. The collected soil should be contained near the actual field to take into account any *in situ* environmental factors.

Section II

Cyst movement by cultivation operations

Chapter 3

Movement of cysts by cultivation operations

3.1 Cultivation operations in UK potato production

In the UK, most maincrop potato production occurs within a crop type rotation. Rotation lengths vary depending on a number of factors such as soil type, pests, diseases, weeds and local environmental conditions. A survey conducted by Minnis *et al.* (2002) found that the mean length of rotation in England and Wales is one in five. Potatoes traditionally follow two cereal crops to provide a suitable break (Anon., 1999). Crop rotation is used as a management technique to help prevent the build up of pests and diseases (see section 1.8) and to allow for the control of weeds. Other crops grown within a potato rotation include cereals, legumes, oil seed rape and sugarbeet.

The cultivations adopted during a crop rotation are dependent on several factors such as soil type, crop grown and cultivation machinery available. Cultivation operations are used within tillage systems. Tillage has been defined as 'any mechanical manipulation of soil carried out for the purpose of nurturing crops and subsequent soil manipulation that takes place during the crop' (Anon., 1993). In addition to cultivations within a tillage system, soil manipulation can also occur at harvest for root and tuber crops.

Specific cultivation operations can be separated into primary and secondary tillage. Primary tillage practices are normally the deepest operations used to loosen the soil and mix previous crop residues. The cultivation machinery used for primary tillage are ploughs and sub-soilers. Secondary tillage is used to prepare a fine tilth and uniform seedbed. Machinery usually classed as secondary tillage equipment includes tines, discs, harrows and bed-forming implements.

The tillage systems commonly adopted to establish crop in the UK are conventional tillage, minimum tillage, direct drilling and bed-forming tillage. Conventional tillage usually includes primary and secondary cultivation operations. Minimum tillage can also be referred to as reduced tillage, a system that uses the least amount of soil manipulation to prepare a seedbed. Direct drilling systems do not utilise any cultivation operations prior to

drilling. Bed-forming tillage systems involve the creation of ridges or beds into which the seeds or tubers are planted; this system is usually adopted for root and tuber crops.

3.2 Movement of soil by cultivation operations

This section reviews research on the movement of soil by cultivation. This includes the horizontal movement of seeds, pests and diseases by cultivation practices. It focuses on the techniques employed for monitoring movements and the construction of models for within-field soil movement by cultivation.

3.2.1 Soil movement

The movement of soil by tillage systems is an important factor in relation to soil erosion and soil conservation strategies. For this reason it has been widely studied using a number of different techniques.

One widely used technique involves the use of fallout Caesium-137 (^{137}Cs) to assess the patterns of soil movement and deposition in watersheds (Ritchie and McHenry, 1975; McHenry and Bubenzer, 1982). ^{137}Cs was produced and dispersed in the environment during atmospheric testing of nuclear weapons in the 1950s and 1960s, and was subsequently deposited on the land by precipitation. Due to the strong adsorption on soil particles, any change in ^{137}Cs levels is proportional to that of the topsoil. The distribution of ^{137}Cs in both horizontal and vertical planes across a landscape shows the spatial patterns of net soil movement within fields and entire watersheds. This technique has been widely adopted in North America for studies investigating the movement of soil by tillage (McHenry and Bubenzer, 1985; Cao, *et al.,* 1994; Lobb, Kachanoski and Miller, 1995; Govers *et al.,* 1996; Montgomery *et al.*, 1997; Lobb, Kachanoski and Miller, 1999; Lobb and Kachanoski, 1999; Lindstrom, Schumacher and Schumacher, 2000).

A similar technique was employed by Sibbesen (1986) in long-term field experiments using phosphorus (P) levels to measure soil movement by tillage. A number of factors for the construction of a model using P needed to be added. The P levels had to be considered in terms of annual dressing, removal by crops and the annual transport of P from the plough-layer to the subsoil by leaching and up by plant roots and soil animals. Once these factors were given values the net movement of P in and out of plots was attributed to soil movement by cultivation over time. Due to uptake of P by plants and other soil organisms within the plots, the accuracy of the changes in P as a tracer for the net soil movement within the plots is problematic.

Other studies have placed objects within the soil and then measured the distances they were moved by tillage. Thapa, Cassel and Garrity (1999) placed granite rocks (3 to 4 cm diameter) in the soil prior to tillage and used them as soil movement detection units. Plastic beads have been used as tracers in soil tillage studies where after the tillage operations a profile was excavated to reveal the movement of the beads (Sharifat, Kushawaha and Reed, 1994; Spoor and Fry, 1983) . Soil profiles have been used in other studies to provide evidence of movement of tracers, such as Govers *et al.* (1994) who used a profile to show the movement of plastic spheres (15 mm in diameter) with a metal core. Aluminium cubes (15 mm) have also been used as tracers where they were recovered by careful excavation of the trial site post tillage (van Muysen *et al.*, 1999; van Muysen and Govers, 2002). These studies are highly intensive as the post tillage excavation is very slow and labour intensive to ensure accurate recovery of the tracers.

A number of studies used metallic objects because they can be located using metal detectors. One such study by de Alba (2001) used labelled hexagonal nuts (10 mm in diameter), buried at 15 cm in a grid. Post-tillage, they were recovered and from the labelling their movement was determined.

The movement of tracers in all the studies were then equated to the mass movement of soil by tillage. These studies using different-sized and -shaped objects as tracers to investigate soil movement may not provide accurate data about the distance that soil is moved by tillage, as tracers may not accurately replicate the behaviour of soil particles when moved by cultivation practices.

The techniques employed were used to provide data for the universal soil loss equation (USLE) for a given field or watershed. The USLE has been used to guide the selection of soil management practices. This has been subsequently updated to the Revised Universal Soil Loss Equation (RUSLE) computer program (Renard *et al.*, 1994). However this computer program does not provide spatial patterns and so recently, new simulation models have been constructed to provide this additional information. These models focus on soil movement on hill-slopes to predict soil mass losses (Lobb and Kachanoski, 1999; Muysen *et al.* 1999; Lindstrom, Schumacher and Schumacher, 2000; Torri and Borselli, 2002).

3.2.2 Seed movement

As with PCN, the distribution of weed seeds is spatially heterogeneous in fields. The principal source of seed movement and redistribution within the soil is by cultivation (Roberts, Chancellor and Thurston, 1977). Few studies have focussed on the horizontal movement of seeds by cultivation (Howard *et al.* 1991; Rew and Cussans 1997; Grundy, Mead and Burston, 1999; Marshall and Brain, 1999), whilst most have principally investigated the vertical distribution as this is important for both seedling emergence and the seed bank (e.g. Froud-Williams, Chancellor and Drennan, 1983; Moss, 1988).

Rew and Cussans (1997) investigated the horizontal movement of seeds by different cultivators, using barley (*Hordeum vulgare* L.), field bean (*Vicia faba* L.) and oilseed rape (*Brassica napus* L.). The seeds were buried 0.1 m deep prior to cultivation. Four different

tine implements (straight-, flexi, spring-tine and rotary power harrow), a mouldboard plough and seed drill were used in this experiment. The study investigated the effect of the individual cultivations within a standard cultivation sequence, which was followed by a rotary power harrow attached to a drill. No seeds were observed further than 5 m in the direction of cultivation and 0.2 m behind. The smaller oilseed rape seeds were found to be moved further than the barley or field bean seeds. The type of tine used was found to significantly affect the mean movement of the seeds, with the flexi- and spring- tined machines moving the seeds further than the straight tine and rotary power harrow; all were moved less than 1 m. The seed movement was determined by counting the number and distance that seedlings emerged post cultivation. This experiment did not take into account any seed movement below germination depth, or potential lateral movements by the cultivations.

Coloured plastic injection mouldings (cylindrical, 1mm diameter and 3mm long) were used as substitutes for seeds along with barley and tricicale seeds by Marshall and Brain (1999) for their study of horizontal movement. Different coloured mouldings and seeds were placed on the soil surface immediately prior to each of the five cultivations; ploughing, flexi- and straight- tine operations, harrowing and drilling. The movement of the beads was measured using quadrats of 0.01 cm^2 in eight directions (N, S, SW, SE, NW, NE, W and E) in relation to cultivation direction defined as North. Cores (20 cm deep) were also taken at 0.3 m intervals in all directions. Ploughing and drilling were found to move the seeds the least distance, and the mean distance moved by the beads were 0.36 m and 0.26 m respectively. The tine cultivations moved the beads 0.71 m and 1.21 m, while harrowing moved them the most to a mean distance of 1.58 m. They concluded that sequences based on mouldboard ploughing are likely to limit seed movement in soil due to the inversion of the soil, which buries surface seeds, although this is probably dependent on whether the seeds are vertically uniform prior to ploughing. From their findings Marshall and Brain

(1999) constructed a probability distribution function (p.d.f) of the distance a seed will be moved by a cultivation operation. The movements found by Marshall and Brain (1999) are greater than those found by Rew and Cussans (1997).

Grundy, Mead and Burston (1999) undertook a similar study burying different coloured beads at different depths, in the same location, followed by cultivation. The cultivation operations employed were a rotavator, a spader, a spring tine and a power harrow. The sampling was carried out in the same directions as the cultivation, three cores were taken (8.5 cm diameter and 18 cm deep) with the central core being the middle of the plot. The cores were taken at 0, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0 and 4.0 m ahead of the application point and 0.25 and 0.5 m behind. They found no significant movement of seeds by the spring tines or spader; the rotavator caused the most backward movement; the power harrow had the greatest capacity to move the seeds forward over 0.5 m. No lateral movement of seeds was investigated. Sampling every metre after 1 m may have resulted in beads being missed past the application point; movement at these distances was found by Marshall and Brain (1999) using similar cultivation machinery, but using a more intensive sampling strategy.

Truscott and Gilligan (2001) developed a model using the data from Marshall and Brain (1999) to predict the spread of rhizomania in sugar beet crops. The causative agent of this is beet necrotic yellow vein virus, which is transmitted by the fungus *Polymyxa betae.* The fungus spread in soil as cystosori, which are 50 to 100 μm diameter. Soil borne diseases, as for seeds, are found to have a patchy distribution in fields. Using the data from seed research to construct a model for the spread of fungi may not be accurate due to their size differences. They also suggested that the model could be adjusted to predict the spread of other pathogens such as cyst nematodes. Although the concept of using this model for predicting the spread of other pests and diseases is possible, the assumption that these will behave the same as seeds may not be valid. As with any simulation model the information provided will be more robust if the number of assumptions is reduced. This may require

actual information of movement for a pest or disease to be determined and used within the structure of the model.

3.2.3 Cyst movement

Although cultivation is widely accepted as the primary factor affecting the spread of potato cyst nematodes within a field (e.g. Been and Schomaker, 1996; Been and Schomaker, 1999; Been and Schomaker, 2000; Riding and Parker, 2000), few studies have been undertaken to quantify the movement of cysts by cultivation operations. Been and Schomaker (2000) sampled a field intensively in the Netherlands and located the PCN foci present. The foci were then more intensively sampled and found to be long and thin, following the direction of cultivation. Boag, Filipe and Neisten (2000) carried out a similar study looking at the spatial distribution of PCN in a potato field in Scotland. They found that the PCN foci were more circular in shape, and suggested that this was probably due to the farmers cultivating potatoes at 90^{o} perpendicular to the cultivation direction of other crops within a rotation.

In the US, extensive research has been conducted on the impact of tillage on the soybean cyst nematode (SCN), *Heterodera glycine* (e.g. Tyler, Overton and Chambers, 1983; Koenning, Schmitt, and Barker, 1995; Gavassoni, Tylka and Munkvold, 2001). Gavassoni, Tylka and Munkvold (2001) investigated the changes in aggregation of SCN under different tillage systems during soybean production. Soil samples were extracted in contiguous quadrats and spatial patterns mapped using geostatistics. Four different tillage systems were examined; conventional, reduced, ridge (bed-forming), and no-tillage. The results found that no-tillage and ridge tillage promoted aggregation of SCN population, whereas conventional and reduced tillage resulted in a less aggregated spatial pattern. Other studies have used soybean crop yield in conjunction with SCN spatial distribution as

a means of determining the aggregation of SCN (Tyler, Overton and Chambers, 1983; Koenning, Schmitt, and Barker, 1995).

Although tillage systems have been studied in terms of investigating their potential for cyst aggregation, no studies have investigated the distances and directions that the cysts are spread by cultivation operations. There have been no studies similar to those carried out for weed seeds for cysts, where objects were added to the soil prior to cultivation and the distance they moved determined. The experiment carried out in Chapter 4 investigates the movement of PCN cysts by cultivation operations employed during a crop rotation.

3.3 Cultivation operations carried out in potato rotation

This section describes the cultivation machinery investigated in Chapter 4. The cultivations are those traditionally employed in a UK potato rotation (Anon, 1999). These operations include the primary cultivation of ploughing, secondary cultivations, potato planting and harvesting.

3.3.1 Ploughing

Ploughs are usually used as the primary cultivation practice for seedbed preparation, in conventional tillage systems. Two types of plough were used in the cultivation experiment, chisel (Plate 3.1) and reversible mouldboard plough (Plate 3.2).

Chisel ploughing consists of pulling heavy cultivator tines through the soil. There is no soil inversion. The working depth of the chisel plough is normally the same or deeper than that of the mouldboard plough (>30 cm). Six tines were fitted to the frame of the chisel plough used for the experiment.

Mouldboard ploughs are more commonly used than chisel ploughs. The plough acts by slicing into the soil at an angle, creating a furrow. This action fractures and granulates the soil, while inverting it into the previous furrow and buries surface trash. The reversible

function allows the plough to continue the furrows in the same direction once the tractor turns after each run. The depth of ploughing is controlled hydraulically from within the tractor.

Plate 3.1 Chisel plough

Plate 3.2 Mouldboard plough

3.3.2 Potato crop cultivations

Most maincrop potatoes grown in the UK are grown in raised beds, with potatoes planted in two adjacent rows. This system was used as the basis for the potato cultivation sequence undertaken in the experiment in Chapter 5 (Figure 3.1). Potatoes can also be grown on the flat where no bed is formed prior to planting. This tends to be carried out on soils with few stones and limited clod formation potential.

The field is first ploughed (Plate 3.2), to a depth of approximately 30 cm prior to forming potato beds.

Beds are formed to allow the seedbeds to be de-stoned and de-clodded prior to potato planting. Commercial bed-formers consist of two or four ridging bodies. The bed-formers with two ridging bodies form one complete bed and two half-beds each pass. The ridging bodies are double mouldboards, which operate at the depth of ploughing and lift soil into beds either side of the ridging body to form furrows (Plate 3.3). This results in the beds being about twice the height of the ploughing depth.

The bed-tiller is used to break up slabs of wet soil or clods in the bed, and assist soil in drying prior to de-stoning. This operation may not be required on lighter, dry soils due to them having limited clodding potential. The bed-tiller consists of 32 mm diameter round steel spikes, which are driven by the power take-off (p.t.o.) shaft of the tractor. The action of the bed-tiller results in the flattening of the beds; they are fitted with a bed forming kit at the back to reform the bed after tilling (Plate 3.4). Bed-tillers can have operating widths of up to 4 m, which would be used to till two beds per pass.

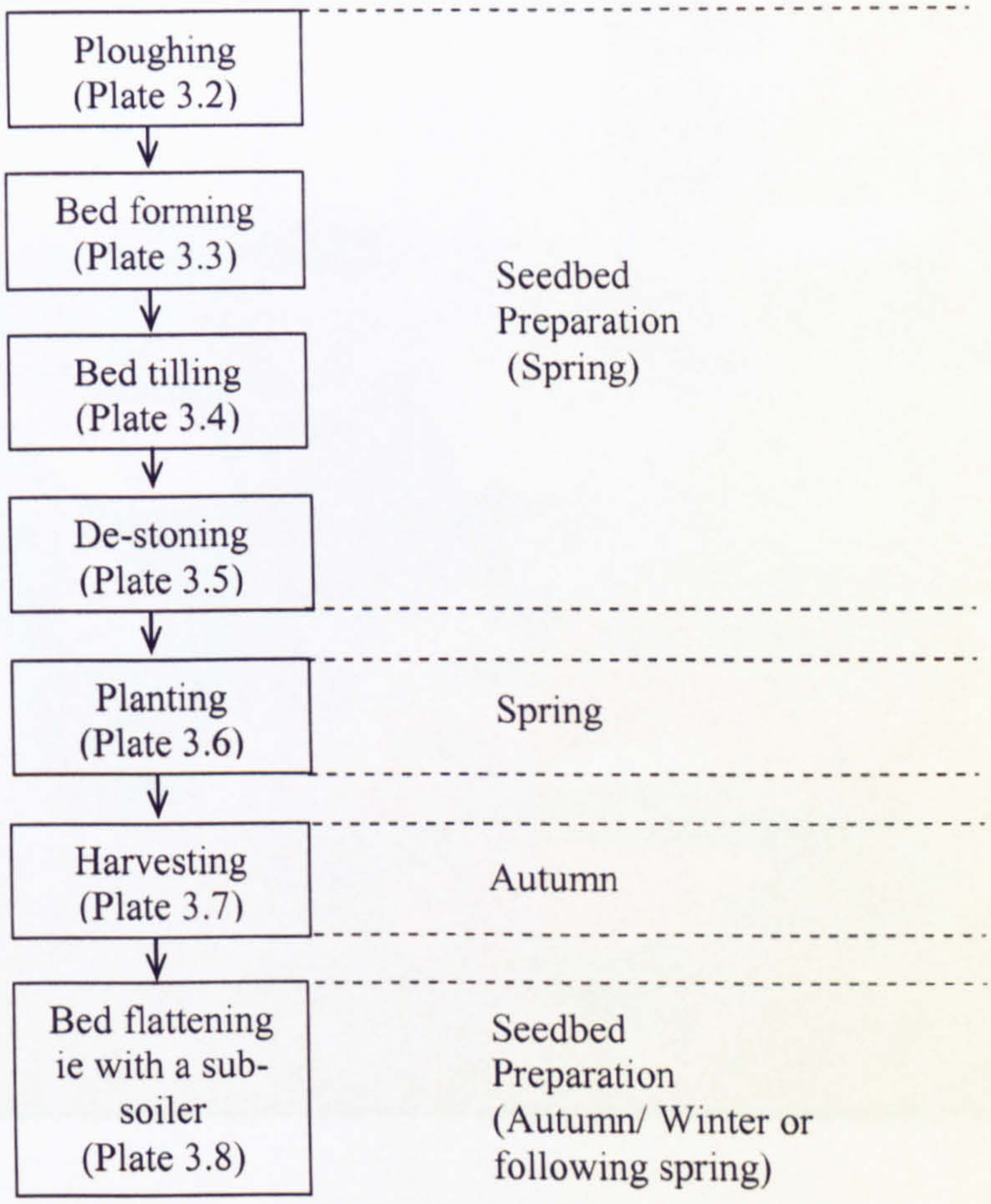


Figure 3.1 Maincrop potato cultivation sequence.

Plate 3.3 Bed-former in operation (source: Grimme (UK) Ltd).

Plate 3.4 Bed-tiller with ridging body kit. Inset- steel rotor spikes of the bed-tiller (source: Grimme (UK) Ltd).

The beds then have the stones and remaining clods removed using a de-stoner (Plate 3.5) to reduce potential mechanical damage tubers at harvest. The de-stoner consists of a front roller or Diablo to flatten the soil in the bed. Share blades then cut into the bed, down to plough depth, and all the soil in the bed is then lifted onto chain webs or combi-stars. As the soil moves up the web (or stars) the soil is sieved back into the bed, whilst the clods are either broken down or remain on the machine with the stones. The stones and clods are then dropped into the furrows, or a trailer, at the side of the bed by a cross conveyor.

Plate 3.5 De-stoner with diablo rollers and webs. Inset- diablos for de-stoner (source: Grimme (UK) Ltd)

Automated potato planters can be set for one to three rows per bed (Plate 3.6). Maincrop potatoes are normally planted with two rows per bed. The planter has narrow furrow openers that operate in the bed, which can be adjusted depending on the planting depth (normally approximately 15 cm). After the furrow is opened the seed-tubers are dropped into the bed at set adjustable intervals. At the back of the planter the shaping board and ridging bodies fill in the narrow furrows containing the tubers and re-form the bed.

The next soil cultivation operation takes place at harvest by a mechanical potato harvester (Plate 3.7), which acts in a similar manner to the de-stoner. The front of the harvester has a roller or diablos which flatten the bed and share blades that cut into the bed. The soil is

lifted onto web conveyors where it is sieved back into the bed; the potatoes are collected in a trailer moving adjacent to the harvester.

Plate 3.6 Potato planter, planting on the flat.

Plate 3.7 Potato harvester with diablo rollers and webs (source: Grimme (UK) Ltd).

Post harvest, and prior to the next crop in a rotation, the potato beds are flattened. This can be achieved by several cultivation practices such as sub-soiling, terra-discing and spring tining. The cultivation is performed along the beds, breaking up the soil and resulting in lateral dispersion from the higher soil in the bed down into the furrows.

A sub-soiler (Plate 3.8) is normally used to penetrate to deeper depths than conventional cultivators in order to break up layers of soil that have become compacted by the movement of heavy machinery or as a result of ploughing at the same depth. Normal working depth for a sub-soiler is approximately 50 cm (Shippen, Ellen and Clover, 1987). For the purposes of bed flattening the working depth is reduced. The sub-soiler used in the experiment (Chapter 4) had five tines and a crumbler roller.

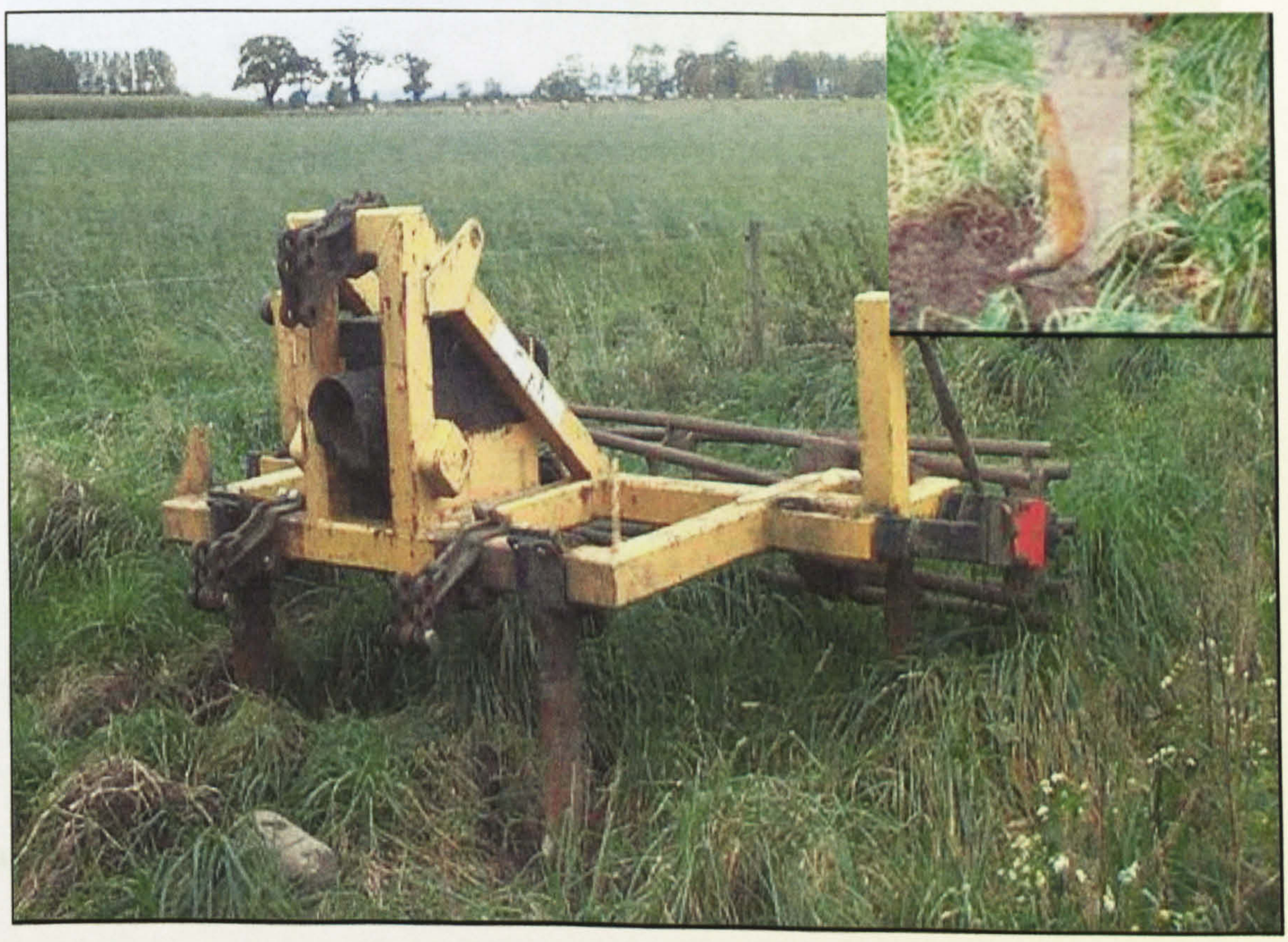


Plate 3.8 Sub-soiler with crumble roller. Inset- fixed tine of sub-soiler.

Disc cultivators can be used for primary and secondary cultivations depending on the size of discs and therefore its working depth. The terra disc used was a Smaragd 8 (Plate 3.9), which consists of two rows of wide-wing shares and a row of angled and concave discs with two tube bar rollers. They are usually employed to prepare a seed bed for cereals by breaking up the soil and crop residue and flatten the seed-bed. The normal working depth is 10 to 15 cm which is also a suitable working depth for bed flattening.

Spring tine cultivators consist of spring steel tines (usually C-shaped), mounted on a frame (Plate 3.10). The tines judder as they are drawn through the soil, helping to break down clods. The spring tines normally operate at a depth of less than 15 cm, which is also suitable for the bed flattening operation.

Plate 3.9 Terra-disc cultivator.

Plate 3.10 Spring tine. Inset- C- shaped tine.

3.3.3 Cereal crop cultivations

Cereal crops are planted on flat uniform beds in conventional tillage systems. The fields normally undergo ploughing then secondary cultivations prior to the seed drill. The seed drill causes minimal soil disturbance. Growers usually use combination machinery, which consists of secondary cultivators and drills manufactured together to reduce the number of passes over land required. Two such secondary cultivators are spring tines (Section 3.3.2) and power harrow.

The power harrow (Plate 3.11) is widely used for seedbed preparation and is fitted with vertical rotating tines that are driven from the p.t.o. of the tractor. The tines rotate, resulting in the tilth being stirred and clods being broken up. The power harrow has a back roller to flatten the bed after it has been worked by the tines. The speed of the tractor and that of the p.t.o. can be used to alter the fineness of tilth. The power harrow normally operates to a depth of 15 cm.

Plate 3.11 Power harrow. Inset- rotating tines.

Chapter 4

The lateral movement of potato cyst nematodes in beds by bed-tilling and harvest operations.

4.1 Introduction

This chapter investigates the lateral movement of PCN in potato beds by mechanical harvesting and bed tilling operations. This was an initial experiment designed to determine cyst movement using the redistribution of PCN populations in a field. This was viewed as a possible method for determining cyst movement within the field by multiple cultivation operations. The aims of this investigation were to determine whether lateral movement of PCN within a potato bed occurs during the bed tilling and harvesting operations and assess whether PCN populations in fields could be used for subsequent cyst movement studies.

The bed-tiller is designed to break-up the soil in the bed which could potentially result in the lateral movement of cysts. The operation of mechanically harvesting a potato crop involves the lifting of the potato bed and then replacement of the soil in the bed. This operation could also potentially result in the movement of soil both along the bed and laterally within the bed.

4.2 Materials and methods

The experiment was carried out on a field (Swan's Leasow) at Harper Adams University College in the Autumn, 2001. The field was selected on the basis that it was in potatoes and was known to be infested with PCN. Point samples had been taken from the field and their location recorded using GPS, prior to planting, for the siting of other researchers' experiments. From these data it was possible to select the experimental area where PCN were known to be present. The field was planted with the susceptible cultivar Estima. The experimental site was sampled prior to the experiment at 10 m intervals along the potato beds to determine the soil type and soil moisture content. The soil type within the experimental area was sandy loam (Rowell, 1994) and had a total soil moisture content range of 15 to 17 % (Miller and Donahue, 1990).

The experimental site consisted of five neighbouring, double-rowed beds. Along the beds 10 m plots were marked out to produce five plots per bed . The experiment consisted of ten plots to be sampled and fifteen discard plots. The experimental plots were selected using a semi-randomised design, stating two plots to be used per bed (Figure 4.1). To take into account any possible horizontal soil movement by cultivation the 10 m plot length was divided with the middle 6 m section sampled (Figure 4.2).

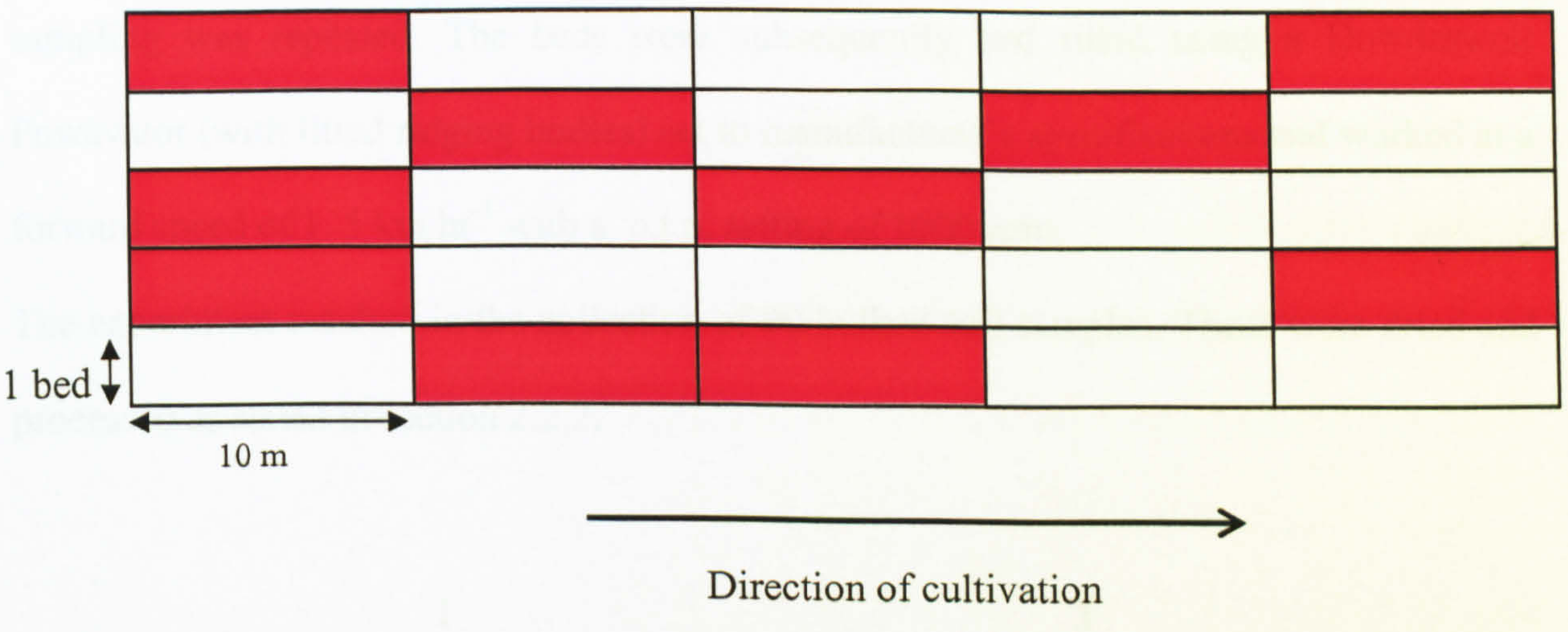


Figure 4.1 Experimental design for lateral movement of PCN by harvest and bed-till cultivations. Plots shaded red were sampled

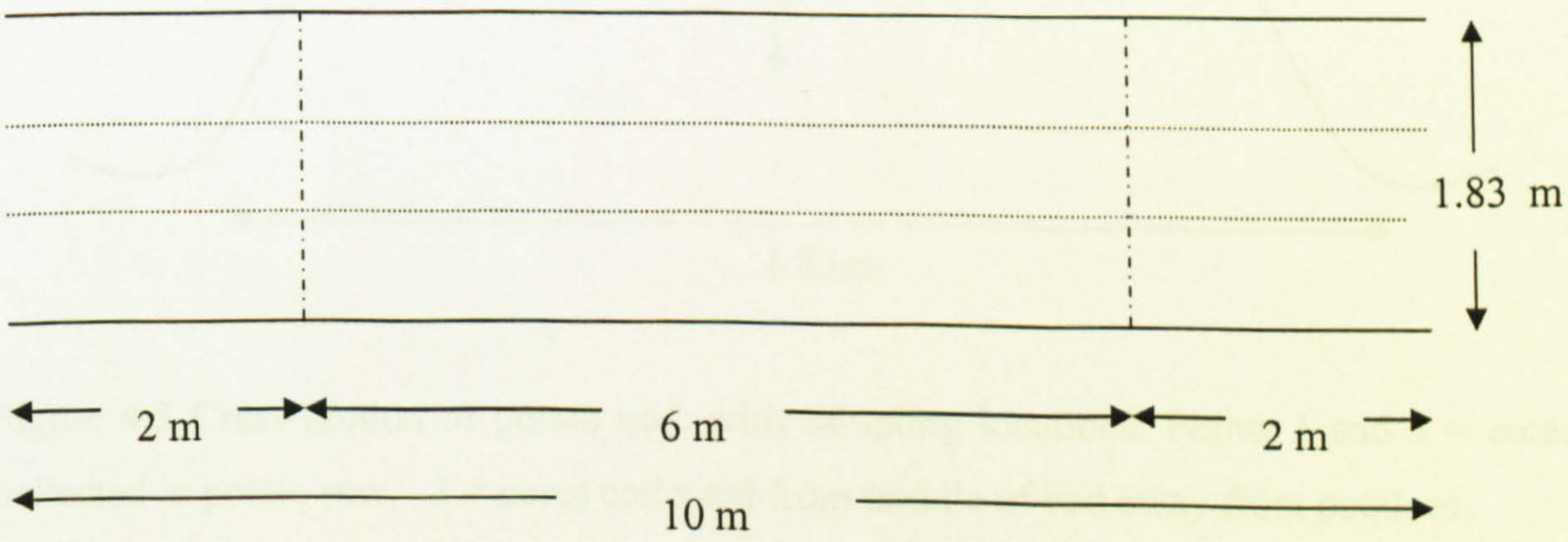


Figure 4.2 Experimental plot for lateral movement of PCN by harvest and bed-tilt cultivations.

Soil samples were collected post cultivation, using a cheese type corer (30 cm length and 2 cm diameter), from three locations across the bed, two in the potato rows and the third from the middle of the bed (Figure 4.3). Each sample consisted of 30 bulked cores, which were systematically collected along the 6 m sample area.

The plots were individually harvested using a Thyregod two-row, web-chain lifter. This was set to the manufacturers specifications, and worked at a forward speed of 1.5 km hr^{-1} with a power take-off (p.t.o.) setting of 540 rpm. This harvester lifted the potatoes and dropped them on the surface of the plot bed. The potatoes were then removed and the soil sampling was repeated. The beds were subsequently bed tilled using a Dowdeswell Powavator (with fitted ridging bodies) set to manufacturer's specifications and worked at a forward speed of 1. 5 km hr^{-1} with a p.t.o. setting of 1000 rpm.

The experiment resulted in the collection of 90 bulked soil samples. These were dried and processed as stated in section 2.2.2.

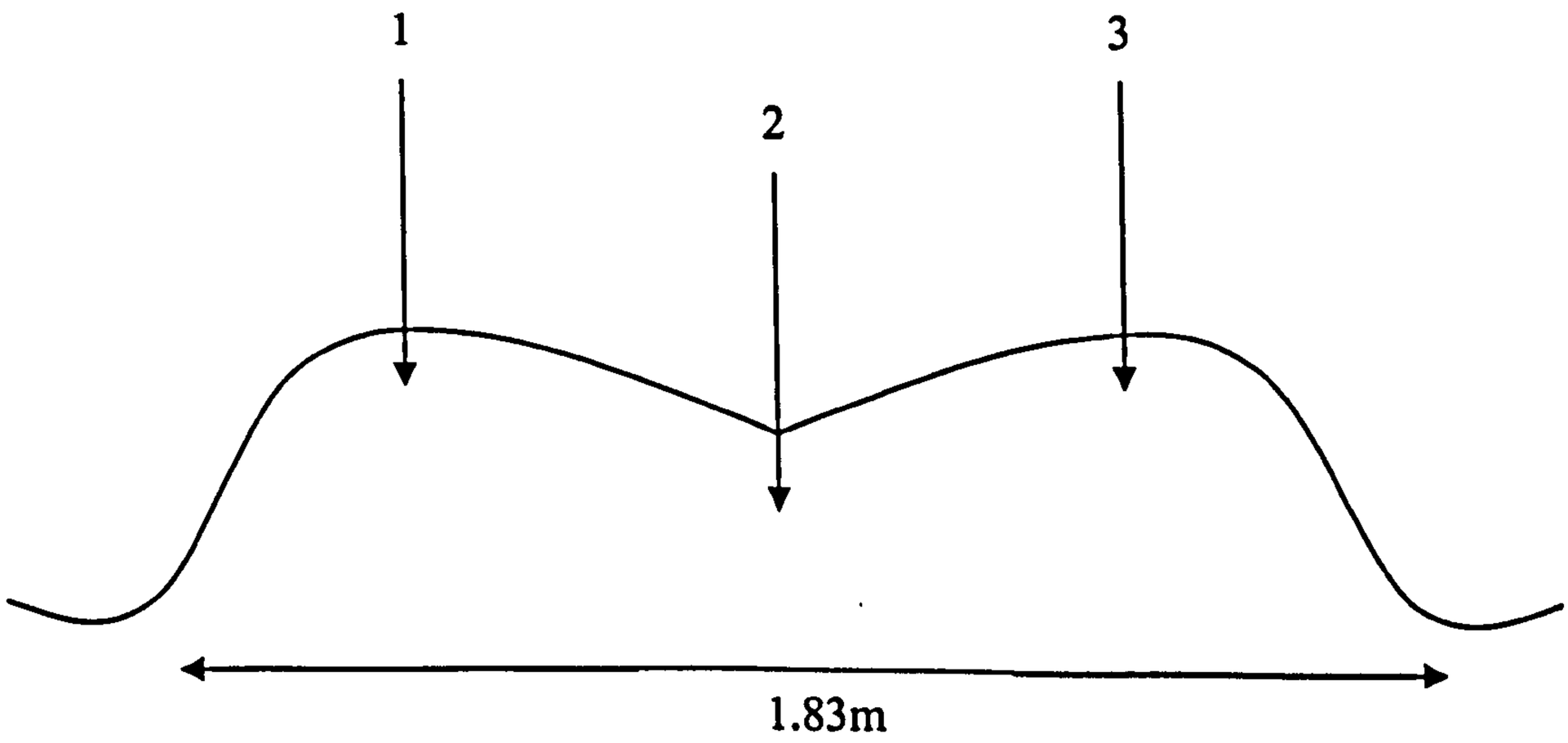


Figure 4.3 Cross-section of potato bed, with sampling locations. Points 1 and 3 = cores collected in potato row, 3 = cores collected from middle of bed away from potatoes.

4.3 Data analysis

The data were converted to proportions prior to analysis for lateral movement. The data did not require transformation and were analysed using a GLM for analysis of variance. All analyses were performed using Minitab 12 (MINITAB INC.).

4.4 Results and Discussion

The PCN population densities were assessed at cyst level to allow for any changes in PCN population densities by cultivation being as a result of cyst movement. Any lateral movement of cysts would result in changes in the proportions of the total cyst counts for the bed found in the sampling points across the bed.

The proportions of cysts found at the sampling points at the three sampling times are shown in Figure 4.4. No significant differences in cyst numbers were found across the bed following the harvesting or bed tilling operations, P= 0.988 and 0.765, respectively.

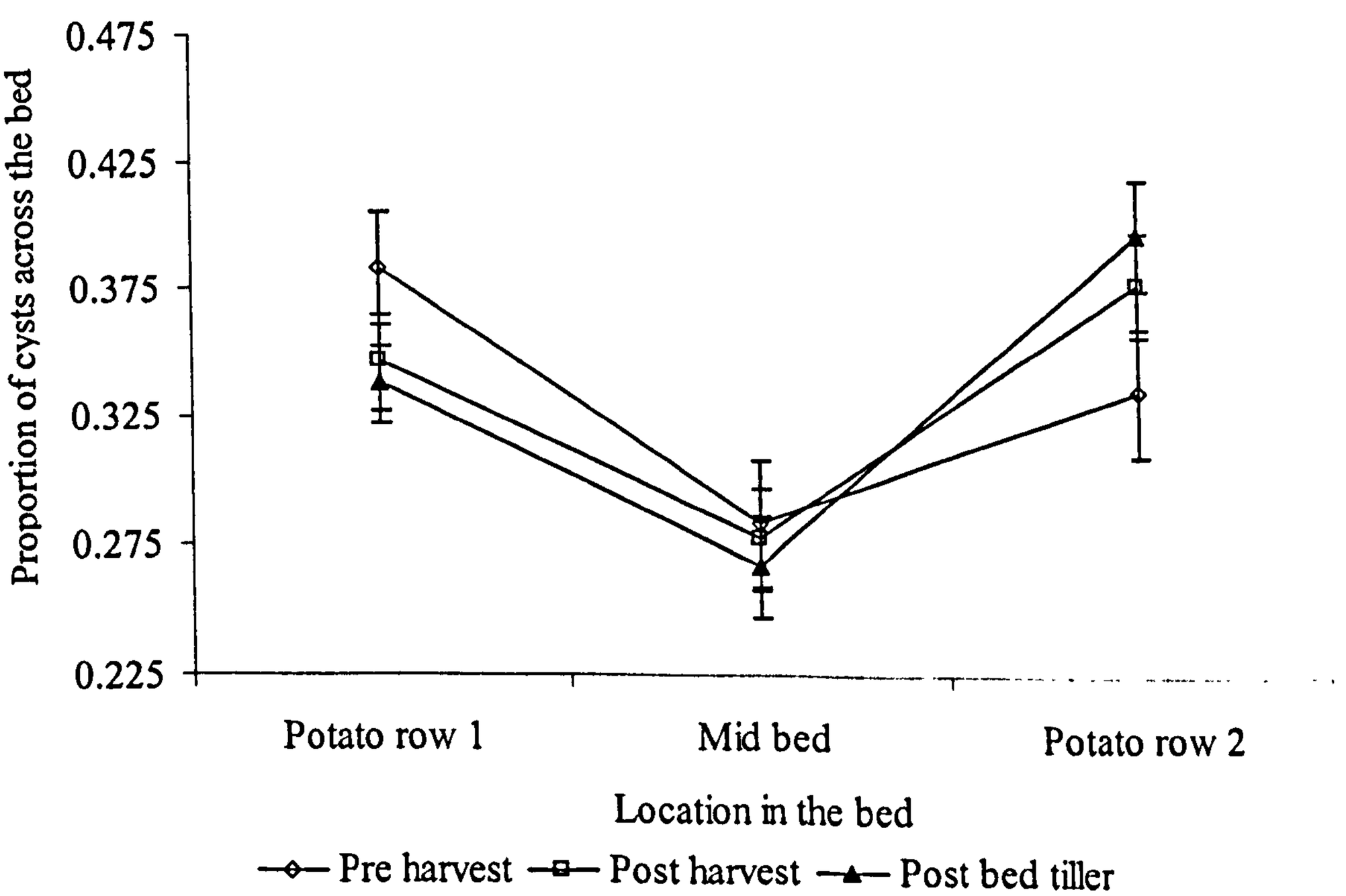


Figure 4.4 Proportional change of cyst numbers across the potato bed after potato harvesting and bed tilling (with +/- SE bars).

An interesting observation is that there is a movement trend across the bed by the two cultivations, suggesting that both result in some redistribution of cysts in the same direction across the bed. Although both cultivation implements are designed to act on the soil in a specific location this shows that some lateral movement does result. For these cultivations the PCN movement appears to be in a specific lateral direction. Implementing a more intensive sampling method across the bed may provide more information on the actual lateral movement of PCN across the bed by the cultivations. However, in terms of the actual movement of PCN within a field by cultivations, any lateral movement of these two operations is likely to be of limited importance. This is due to the lateral movement being restricted to the width of the bed. Additionally the results suggest trends of cyst movement by cultivation not specific distances that a cyst is being laterally moved by the cultivations.

The experiment does show that a PCN population could be used to indicate movements of cysts by cultivation. However, practical problems for the use of this technique in subsequent multiple cultivation experiments exist. This experiment investigated cyst movement along one axis whereas actual movement could potentially result in any horizontal direction depending on the cultivation operation. This would result in the need for intensive point sampling in a grid pattern to determine distances and direction. This level of intensive sampling would be required before and after each cultivation operation. In addition to increasing the sampling size and intensity, the movement of cysts from other locations could obscure any observations of cyst movement from a point sample. For example, if an operation resulted in both movement forward and back, which could potentially result in no net movement being found at given location, which would lead to the conclusion that the cultivation caused no movement of cysts. Gavassoni, Tylka and Munkvold (2001) monitored natural populations to study the differences between tillage systems on the potential for aggregation of soybean cyst nematodes (*Heterodera avenae*).

However, this study did not look at the movement of cysts by cultivation it only looked at the aggregation of the populations when using different tillage systems. This provided information on what could be expected to occur during a crop, but not changes in the cyst distribution by the cultivations required to implement the systems. The sampling of a spatially dynamic natural population in larger cultivation experiments is also problematic in terms of the patchy distributions within a field. This could pose problems with regard to achieving suitable levels of replication of cultivation operations.

The need for intensive sampling and potential for movement from other sampling locations suggest that the use of in-field PCN populations for studies of cyst movement by cultivation is problematic. The intensity of sampling required would restrict the number of cultivation operations that could be investigated.

Chapter 5

The movement of PCN cysts in fields by cultivation operations

5.1 Introduction

This chapter investigates the movement of PCN cysts, which occurs as a result of cultivations during a potato rotation. This involved experiments investigating the movement of cysts by the range of cultivation machinery commonly utilised in potato production and in other crops within a potato rotation. Before this could be undertaken a technique had to be developed to enable cyst movement to be quantified.

5.2 Development of a technique to monitor cyst movement

From the research reviewed in Chapter 3 several possible techniques were identified to monitor cyst movement; the advantages and disadvantages are discussed below. Following the results of the experiment in Chapter 4 the monitoring of natural PCN populations was rejected.

5.2.1 Creating new PCN foci in a field

To study PCN cyst movement within a field the construction of artificial foci was considered. However, there were a number of problems with this method, including field selection and the large number of cysts required. The use of a field already infested with PCN could result in the natural population of cysts being sampled and counted as those from the artificial foci. The use of a field previously uninfested with PCN was not an option, as it would result in the introduction of a PCN infestation. This problem could have been addressed by sterilising the cysts before their addition to the field. Also, for the fields with a natural PCN population the cysts added to the field could have been marked (i.e. by staining) to identify them as not being from the field population.

However, the main constraint to setting up artificial foci within a field was that of the numbers of cysts required. To create artificial foci of cysts for multiple cultivations with replication would require millions of cysts extracted from soil, which is a very time

consuming process. For example to create a 1 m^2 foci to a depth of 0.3 m and a population a PCN population at a density of 1 cyst g^{-1}, in a sandy-loam soil (with a bulk density of 1.5 g^{-1} cm^3) (Miller and Donohue, 1990), would require 200,000 cysts.

5.2.2 Use of a substitute for PCN cysts

Due to the constraints previously mentioned it was decided to use a cyst substitute to simulate cyst movement. From the review of previous movement studies a number of possibilities were examined as potential substitutes. It was concluded that the substitute should be one that had characteristics that closely resembled those of a cyst. Rew and Cussans' (1997) work on the movement of seeds found that different sizes of seed were moved by cultivation in proportion to their size; the smaller the seeds the further they were moved. Thus the cyst-substitute had to be the same size as cysts. Further investigations of seed size and shape revealed that no suitable seed species was available (e.g. Anon., 1986; Holm-Nielsen, 1998; Hanf, 1983). Plastic tracers, similar to those used by Marshall and Brain (1999) were too large and of irregular shape.

In previous work at Harper Adams, fluorescent nematicide microgranules had been used for incorporation studies on nematicides (Woods *et. al* 1999). The microgranules were gypsum, coated with fluorescent paint. They are of a similar shape and size to cysts, but are 10 times the density. Prior to their use as a substitute for cysts an experiment was required to ascertain whether they behaved in the same way as cysts when subjected to cultivation operations.

5.3 Preliminary work

Although, the use of cysts for the cultivation work had been rejected, they were required for an experiment to validate whether the microgranules were a suitable cyst-substitute. A method to compare relative movement of cysts and microgranules in soil was required.

However, the standard method to separate cysts from soil is by flotation, which is not possible for gypsum microgranules.

The microgranules fluoresce under Ultra-violet (UV) light. It was initially thought that a fluorescent seed coating could be used on the cysts for comparison work with the microgranule. However, this would have altered the surface of the cyst and this may an effect on the behaviour of the cyst in soil. Therefore the cysts were altered without altering their surface.

The cysts were first bleached, using a protocol for the bleaching of tree roots with hydrogen peroxide for mycorrhizal detection (Koske and Gemma, 1989). The cysts were placed in 3% hydrogen peroxide for 30 minutes, in a water bath at 80^0C. Leaving cysts for longer than this caused them to rupture. Ragan and Swarup (1986) evaluated stains for the differentiation of cysts from debris. Using their work as a basis, the bleached cysts and then placed in boiling Acid fuschin stain for 3 minutes. This stained the cysts red and made it possible to separate them from soil by eye. Acid fuschin stained cysts were used for the subsequent comparison work.

5.4 Experiment 1: Technique to validate the use of the microgranules as a substitute for cysts

5.4.1. Introduction

The aim of this experiment was to investigate the validity of using a microgranule as a substitute for PCN cysts. The null hypothesis was that there is no significant difference between the behaviour of the nematicide microgranule and that of the PCN cysts when subjected to cultivation operations.

The experiment was carried out in sandy-loam soil in the Soil Hall at Harper Adams University College, Shropshire. The total soil moisture content was 13% (Miller and Donahue, 1990). The size of the facility (1800 m^2) allows the use of field scale operations under controlled soil and environmental conditions.

5.4.2 Materials and methods

5.4.2.1. Preparation of microgranules and cysts

The estimated 30,000 cysts required were bulk extracted from infested field soil; using the Fenwick can method (see section 2.2.2). The cysts were stained using the bleaching and Acid fuschin staining method detailed in Appendix 7.

The microgranules were gypsum particles with a fluorescent pigment coating. Germaines seed merchants prepared the microgranules, the pigment used was Nova Red (Day-Glo©). The microgranules were sieved at 850 μm and 250 μm to ensure that the size range was the same as for the cysts and microgranule (Plate 5.1). Five replicates each of 100 cysts and microgranules were weighed on a microbalance (Table 5.1).

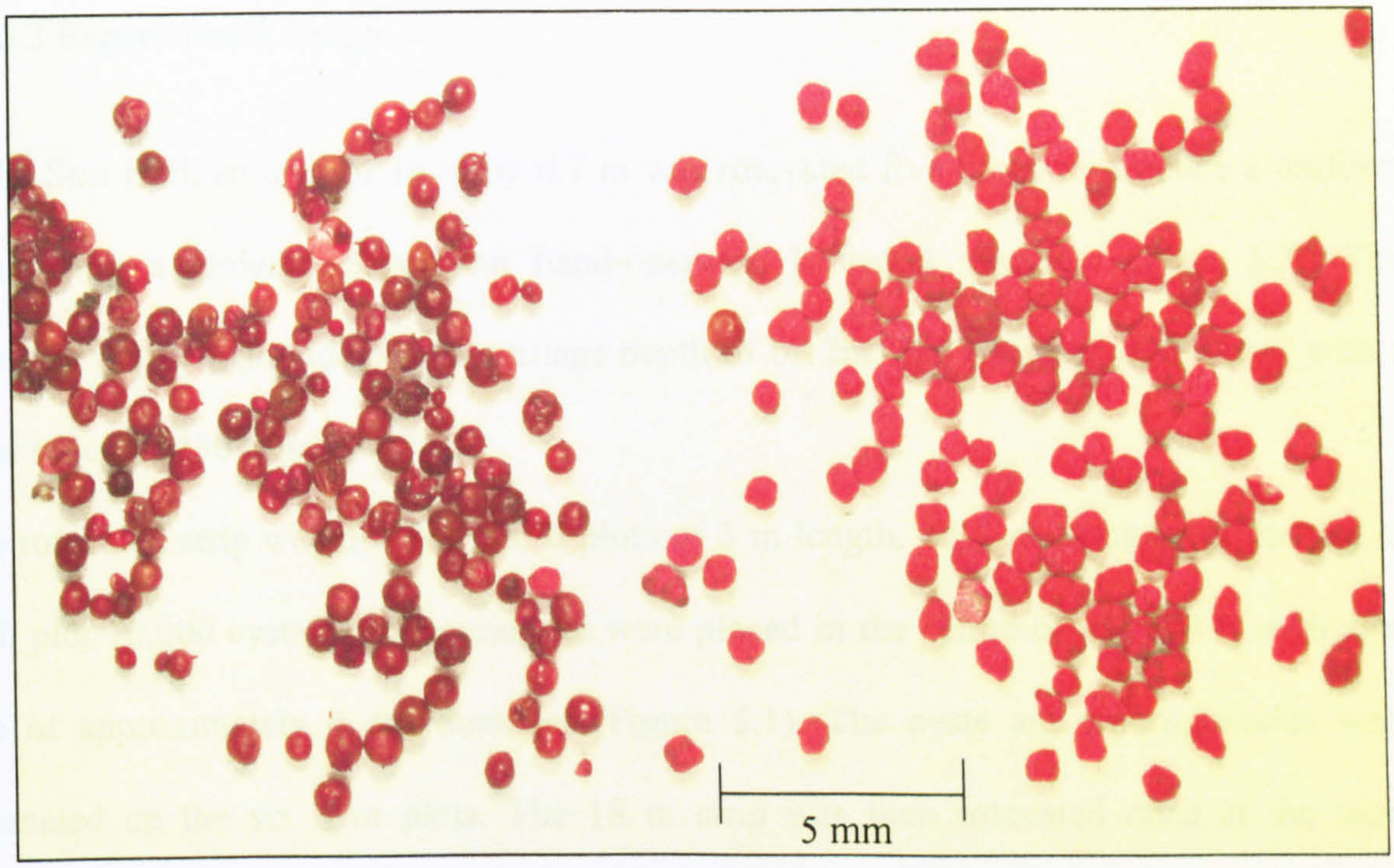


Plate 5.1 Acid fuschin stained cysts and fluorescent microgranules.

Table 5.1 Comparison of cyst and microgranule weights.

Replicate	100 Cysts weight (g)	100 Microgranules weight
1	$2.5x10^{-3}$	$1.94x10^{-2}$
2	$2.0x10^{-3}$	$1.84x10^{-2}$
3	$2.3x10^{-3}$	$1.86x10^{-2}$
4	$2.4x10^{-3}$	$1.78x10^{-2}$
5	$1.8x10^{-3}$	$1.77x10^{-2}$
Mean weight (SE)	$2.2x10^{-3}$ ($1.3x10^{-4}$)	$1.84x10^{-2}$ ($1.84x10^{-4}$)
Mean weight of cyst or microganule	$2.22x10^{-5}$	$1.84x10^{-4}$
Weight of 10,000	0.22	1.838

5.4.2.3 Experimental design

In the Soil Hall, an area of 18 m by 0.7 m was rotavated five times to produce a uniform tilth, using a Howard SuperGem hand-operated L-bladed rotavator (Plate 5.2). The rotavator was 0.7 m wide, set to a tillage depth of 0.17m, and operated at 0.5 ms^{-1} with a rotor speed of 430 rpm.

The rotavated strip was divided into 6 plots of 3 m length. At 1 m along from the end of each plot 10,000 cysts or microgranules were placed in the centre of the plot in a circular pile of approximately 4 cm diameter (Figure 5.1). The cysts and microgranules were alternated up the six 3 m plots. The 18 m strip was then rotavated once at the same rotavator setup as previously described.

Plate 5.2 L-bladed hand operated rotavator (Howard SuperGem). Inset L-bladed tines.

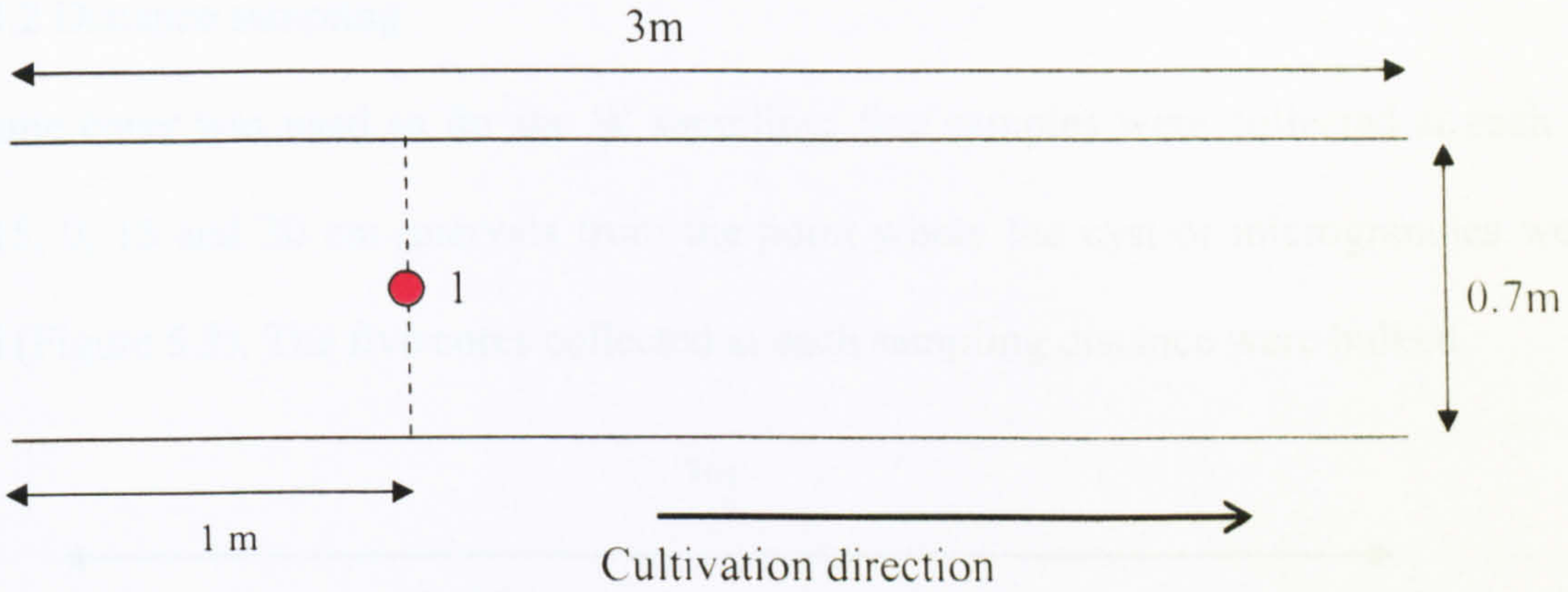


Figure 5.1 Experimental plot design for microgranule validation, Experiment 1 (1 = 10 000 cysts or microgranules).

5.4.2.3 Sampling

The plots were sampled using two techniques.

5.4.2.3.1 W pattern sampling

The plots were sampled using a soil cheese type corer (20 cm depth by 3 cm diameter) in a W pattern. 20 cores were collected in the area 0.5m before the pile of cysts or microgranules and 1m after (Figure 5.2). A template was constructed from card to allow pattern duplication for the 6 plots.

The 20 cores collected in each plot were bulked to produce 1 sample per plot.

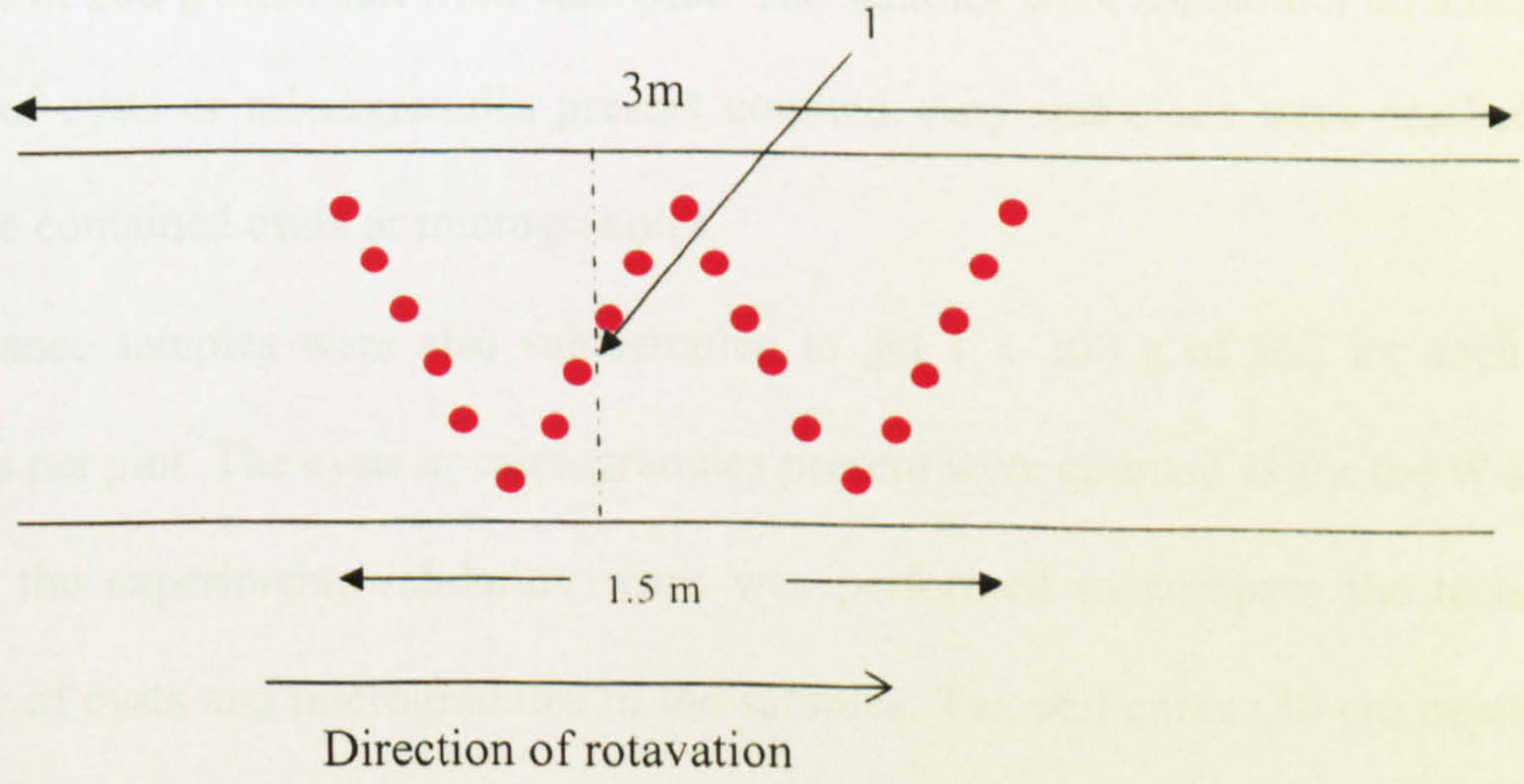


Figure 5.2 W sampling method for microgranule validation, Experiment 1 (1= Location of cyst or microgranule application).

5.4.2.3.2 Distance sampling

The same corer was used as for the W sampling; five samples were collected at each of -30, -15, 0, 15 and 30 cm intervals from the point where the cyst or microgranules were placed (Figure 5.3). The five cores collected at each sampling distance were bulked.

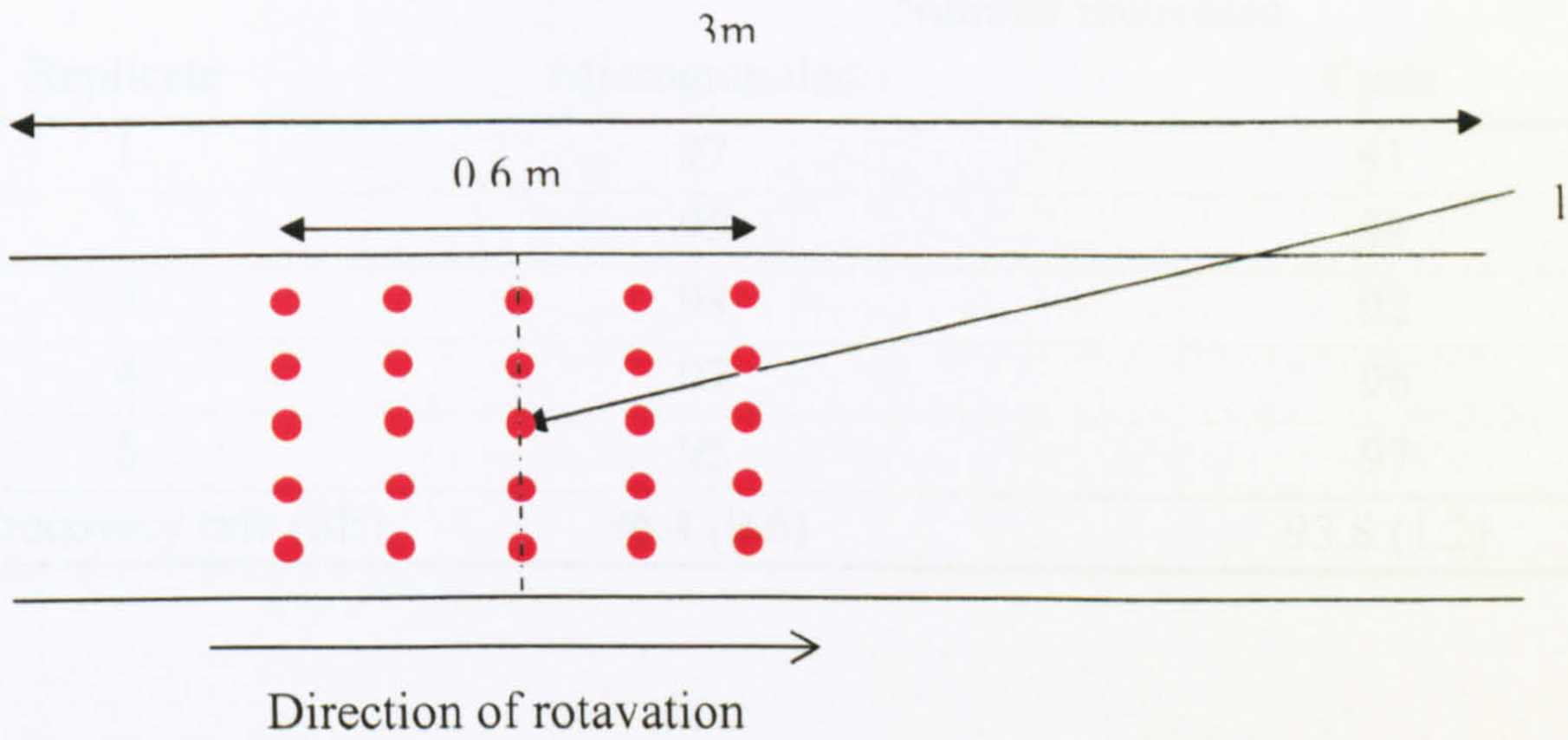


Figure 5.3 Distance sampling method for microgranule validation, Experiment 1 (1= Location of cyst or microgranule application).

5.4.2.4 Sample processing

The soil samples were air dried in an oven at 50^0C for 5 days. The soil was coarsely sieved (4 mm aperture) and mixed thoroughly. Each W sample was sub-sampled to get 3 replicates of 200 g dried soil from each plot. The samples were spread out on a tray and the number of cysts or microgranules present counted. Any soil clods were crushed as they may have contained cysts or microgranules.

The distance samples were also sub-sampled to get 1 x 200 g of soil for each of the 5 distances per plot. The cysts or microgranules present were counted as for the W samples.

Prior to the experiment, validation work was performed to compare the technique for recovery of cysts and microgranules in the samples. Ten soil cores (30 cm depth by 4 cm diameter) were taken from the Soil Hall to produce 10 separate samples. One hundred microgranules were added to five samples and mixed thoroughly; this was repeated with cysts. The samples were processed as for the samples from the main experiment. The

number of cysts and microgranules in the samples was then counted to determine the recovery rate (Table 5.2)

Table 5.2 Recovery rates for cysts and microgranules

	Number recovered	
Replicate	Microgranules	Cysts
1	97	91
2	95	93
3	98	92
4	97	96
5	95	97
Mean recovery rate (SE)	96.4 (0.6)	93.8 (1.2)

5.4.2.5 Data Analysis

Data from the experiment was analysed using analysis of variance (ANOVA) and general linear model (GLM) on Minitab 12 (MINITAB INC.).

5.4.3 Results and discussion

5.4.3.1 W Sampling method

It was found that there was a significant difference between the microgranules and cysts (P= 0.001). Significantly more microgranules were recovered than cysts; a mean of 12.2 microgranules per sub-sample as opposed to 3.4 cysts per sub-sample in their respective plots (Figure 5.4).

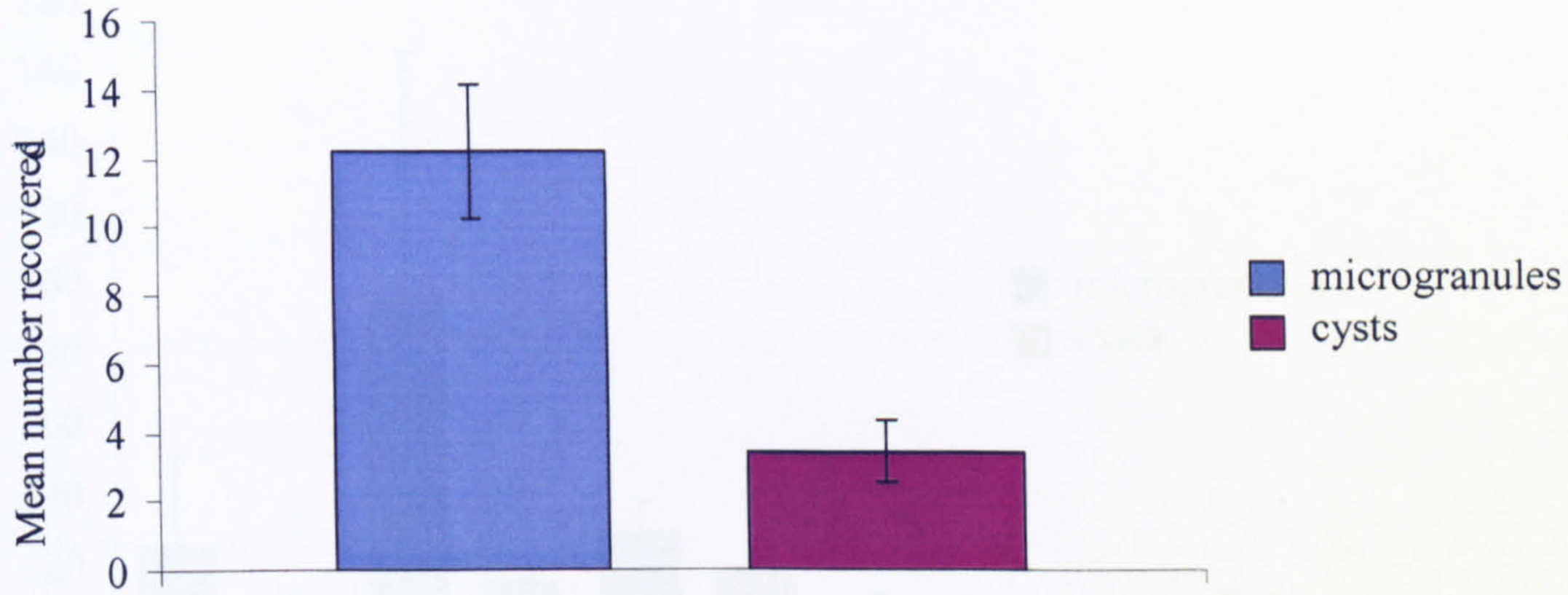


Figure 5.4 Mean number of cysts/microgranules recovered using the W sampling method, Experiment 1 (with +/-SE bars).

Although this sampling method showed a significant difference between the cysts and microgranules, by bulking the cores to produce one sample and then taking 3 sub samples may have obscured the results. This sampling does not show any potential differences in distribution from the application point, which would be of greater use in comparing the behaviour of cysts and microgranules. The use of the W sampling method also resulted in variation of sampling intensity within the plot. However, this was not important due to any variation being replicated for both cysts and microgranules.

5.4.3.1 Distance sampling method

No significant differences between the cysts and microgranules were found (P= 0.889). Although more microgranules were found this was not significant (Figure 5.5). The lack of significance could be accounted for by the differences in recovery rates (Table 5.2). The exception to this was at distance –15 cm, where the sample contained over 250 microgranules. A GLM was performed on the data omitting this sample to see its significance on the results; this did not show any difference to that of the ANOVA.

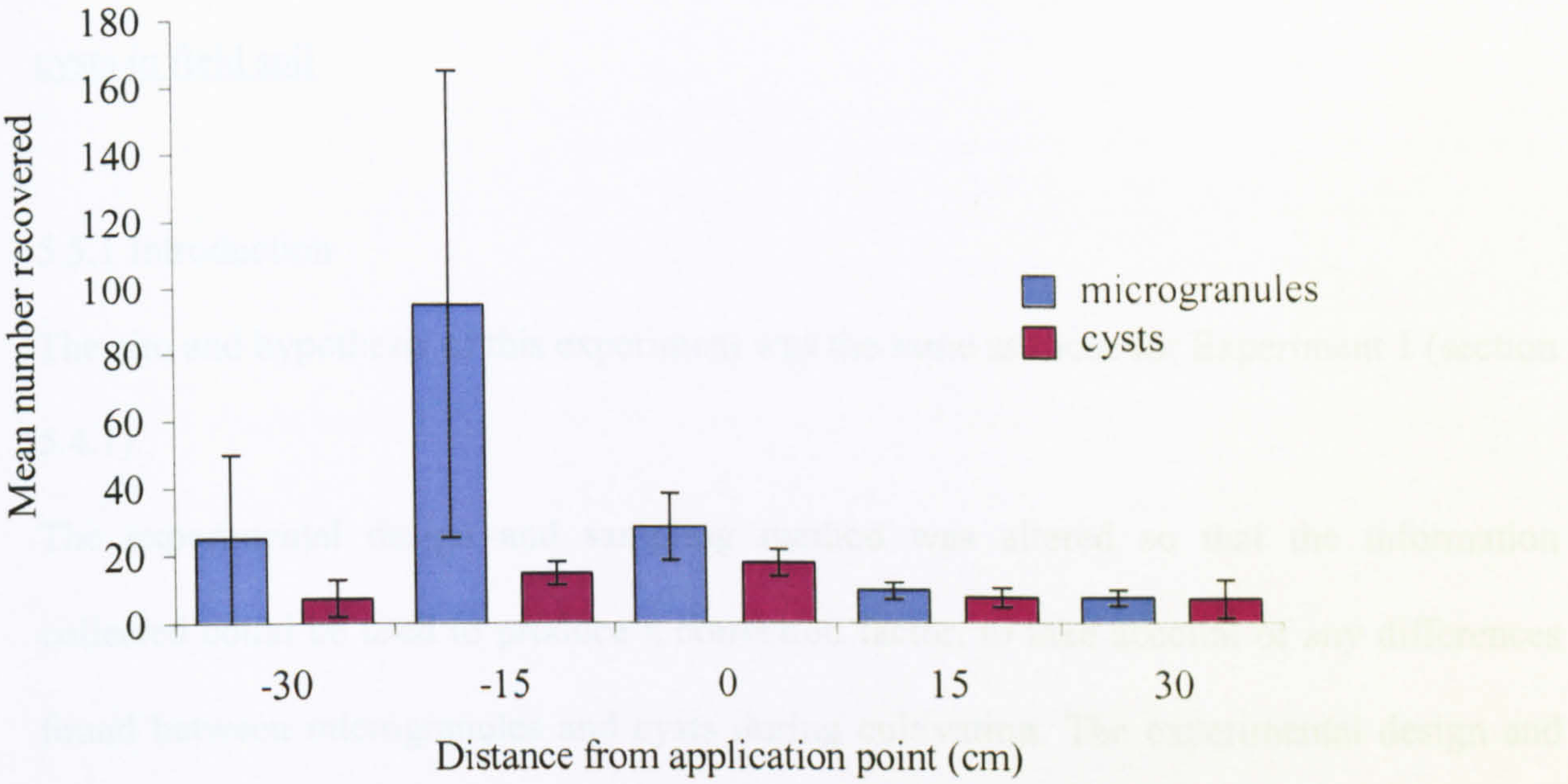


Figure 5.5 Mean number of cysts/microgranules from the application point, Experiment 1 (with +/-standard error bars).

Although the distance sampling showed no significant difference between the cysts and microgranules, the W sampling method showed there was a significant difference. The use of the two different sampling methods within the same plots could have resulted in errors in the distance sampling as it followed the W sampling of the plots. Following statistical advice it was decided that the distance sampling method was the optimum technique for the comparison of the cysts and microgranules. This was due to the W-sampling method not giving distinct movement information within the plot. However, the experimental design needed to be altered to allow greater statistical information to be generated. For this reason a further experiment was carried out.

5.5 Experiment 2: Technique to validate the use of the microgranules as a substitute for cysts in field soil

5.5.1 Introduction

The aim and hypothesis of this experiment was the same as those for Experiment 1 (section 5.4.1).

The experimental design and sampling method was altered so that the information collected could be used to produce a correction factor, to take account of any differences found between microgranules and cysts during cultivation. The experimental design and sampling method was constructed to meet this criterion following statistical advice.

5.5.2 Materials and methods

The microgranules and cysts were prepared as for Experiment 1 (see section 5.4.2.1).

5.5.2.1. Experimental design

The experiment was undertaken in the Soil Hall, away from the location of the first experiment. The total soil moisture content was 13%. The experiment was prepared as for Experiment 1 using the same rotavator (see section 5.4.2.3). A strip of 24 m was rotavated. The strip was divided into eight plots of 3 m length and 0.7 m width, producing 4 replicate plots for the two treatments. The experimental design was a semi randomised block design, a block consisting of 2 plots (Figure 5.6).

One metre into each plot was marked; at this point a line (0.2 m) of 10,000 cysts or microgranules was placed across the middle of the plot (see Figure 5.7).

The strip was then rotavated once, the rotavator settings were as those for Experiment 1.

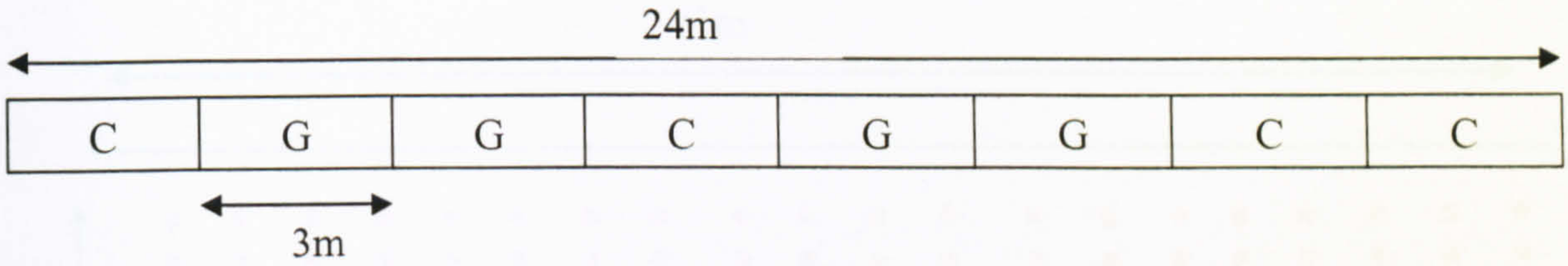


Figure 5.6 Experimental design for microgranule validation, Experiment 2. C= cysts, G= microgranules.

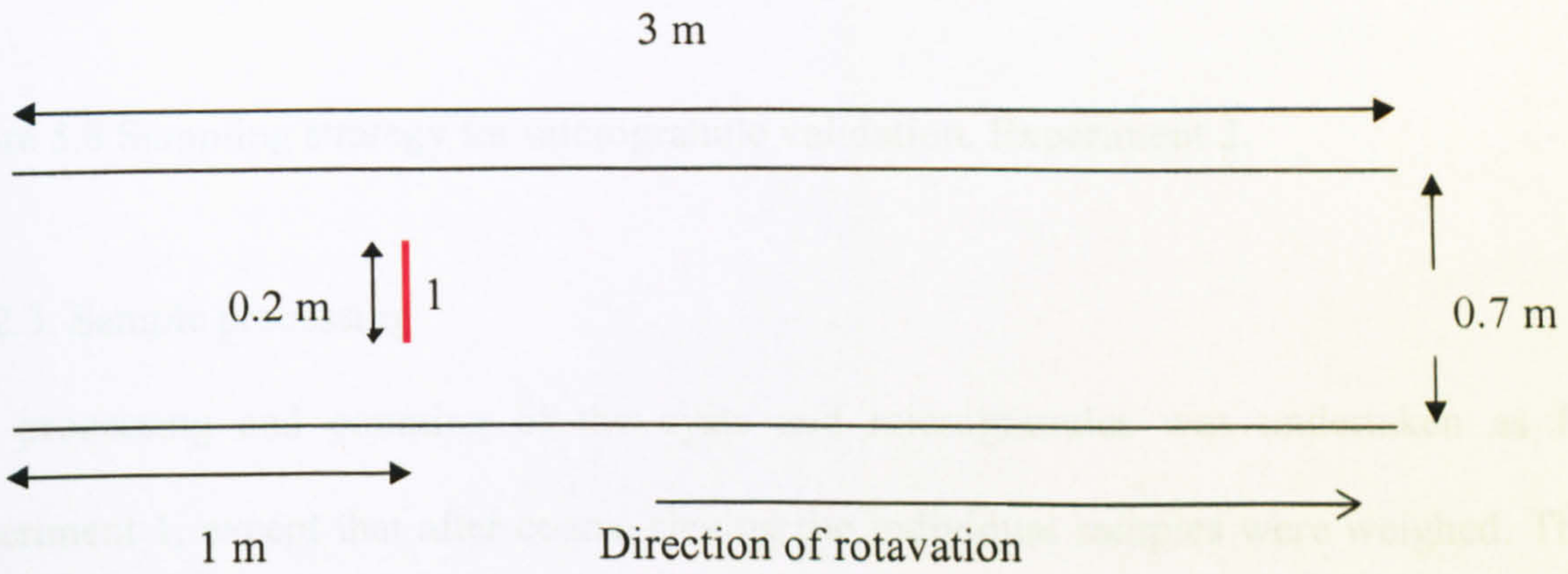


Figure 5.7 Experimental plot design for microgranule validation, Experiment 2. 1 = line of cysts or microgranules.

5.5.2.2. Sampling

The plots were intensively sampled for 1 m length either side of the cyst/microgranule application lines. The length of the plot to be sampled was determined from the results of a previous experiment. A cardboard grid template was constructed to allow samples to be collected at identical 0.1 m intervals within the plots (Figure 5.8).

The point samples were collected using a cheese type corer (20 cm depth and 3 cm diameter); the samples were placed in separate bags. One hunded and five samples were collected per plot.

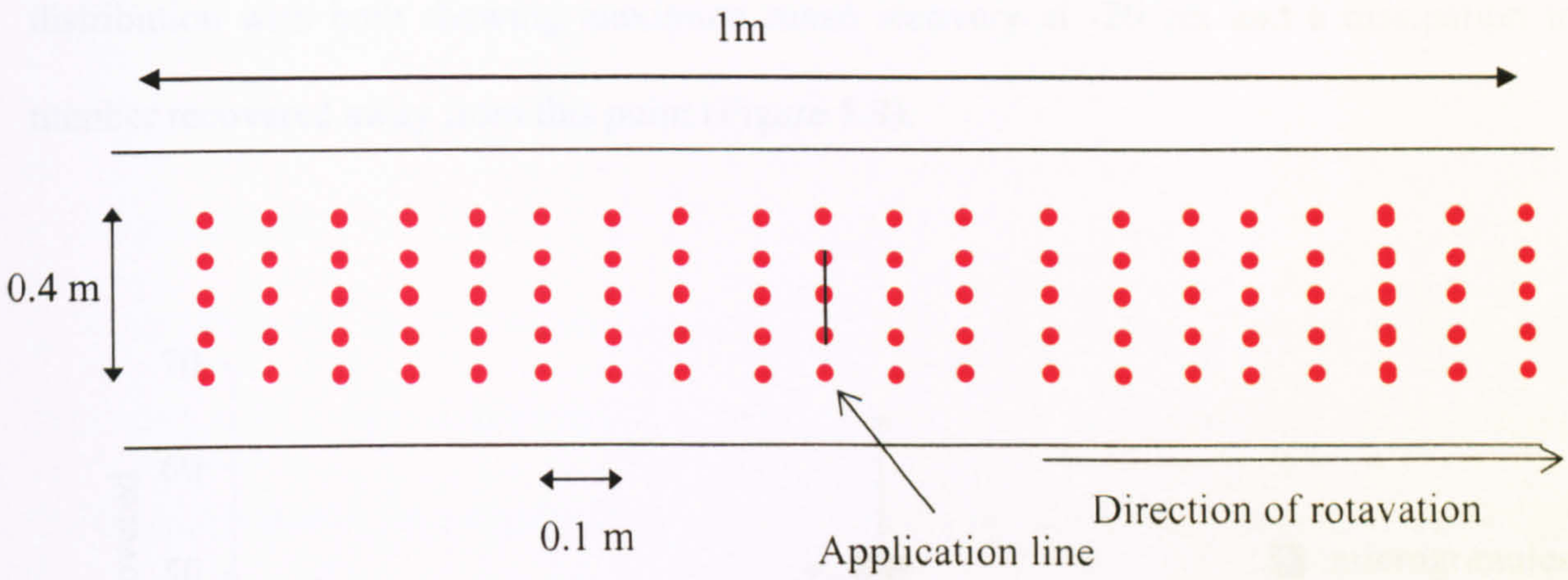


Figure 5.8 Sampling strategy for microgranule validation, Experiment 2.

5.5.2.3. Sample processing

The processing and counting of the cysts and microgranules was undertaken as for Experiment 1, except that after coarse sieving the individual samples were weighed. This was required due to the fine tilth of the plot resulted in soil escaping the corer at sampling. For this reason the volume of the corer could not be used as a standard value for the validation work.

The samples were counted out from the application line and once 3 consecutive lines in either direction failed to have any cysts/microganules present the subsequent samples were not processed.

5.5.2.4 Data Analysis

Data from the experiment was analysed as for Experiment 1 and using the Chi-square test.

5.5.3 Results and Discussion

Following statistical advice it was deemed that the lateral samples at each distance needed to be pooled due to too many values of 0. There was no significant difference between the cysts and microgranules at the distances from the application line (P= 0.599). The cysts

and microgranules present at the distances from the application point formed a similar distribution with both showing maximum mean recovery at -20 cm and a dissipation in number recovered away from this point (Figure 5.9).

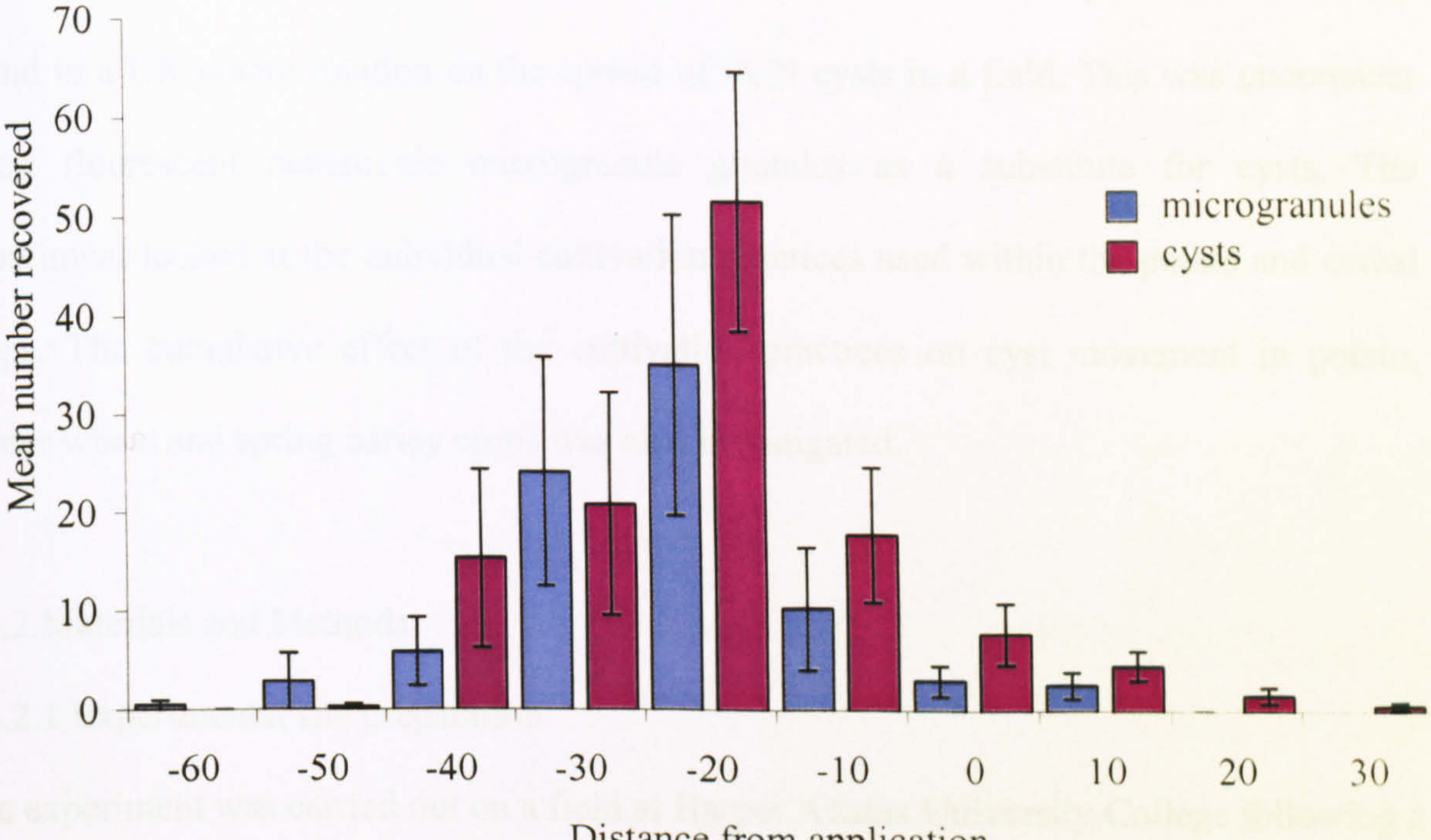


Figure 5.9 Mean microgranules and cysts at distances from the application point, lateral samples at each distance pooled, Experiment 2 (with +/-standard error bars).

Although the microgranules were moved further beyond the application line and the cysts further behind, this was not significant. Subsequent statistical analysis was undertaken to confirm the findings of the ANOVA. Chi-square was used to look at the distribution; fitted to a normal distribution on each plot this showed that the range of means of variance for the cysts was greater than the microgranules, at 5.89-7.53 and 6.68-7.41 respectively. However, the microgranules means lying within those of the cysts suggests that the microgranules are a good substitute for cysts.

Due to no significant differences being found between the cysts and the microgranules, the microgranules were used in the subsequent movement work without a correction factor.

5.6 Horizontal movement of potato cyst nematode cysts in arable soil by different cultivation practices

5.6.1 Introduction

The experimental aims were to determine the effect of cultivation practices commonly found in a UK potato rotation on the spread of PCN cysts in a field. This was undertaken using fluorescent nematicide microgranule granules as a substitute for cysts. The experiment looked at the individual cultivation practices used within the potato and cereal crops. The cumulative effect of the cultivation practices on cyst movement in potato, winter wheat and spring barley crops was also investigated.

5.6.2.Materials and Methods

5.6.2.1 Experimental site preparation

The experiment was carried out on a field at Harper Adams University College following a triticale crop. The experimental site (2 hectares) was ploughed and pressed, using a reversible mouldbourd plough, to break up the previous crop residues. Samples were collected in a 20m grid within the experimental site for soil texture and moisture content analysis. The soil type within the experimental site was sandy loam (Rowell, 1994) with a total soil moisture content range of 9 to 10 % (Miller and Donahue, 1990).

The experimental site was sub-divided into seven areas to investigate different cultivations; the cultivation used were; individual potato crop cultivations, potato cultivations in sequence, bed flattening cultivations, individual cereal cultivations, winter wheat cultivation sequence, spring barley cultivation sequence and ploughing. Each cultivation or sequence of cultivations had three replicates. The length of the experimental plots was determined using information on the movement of seeds by Marshall and Brain (1999)

and was modified according to the number of cultivations in the sequence. The plot width was determined by the width of the cultivation machinery.

The seven sub-divisions of the experimental design within the experiment are descibed individually below. Within the plots, for all the sub-divisions, artificial 'PCN infestations' were added prior to cultivation. A metal tube (20 cm diameter) was inserted into the soil within the plots to a depth of 30 cm. The soil within the tube was then excavated and placed in a cement mixer to which 100 000 microgranules were added. The soil was mixed thoroughly and replaced in the tube and the tube removed. The mixing of the soil was carried out to produce homogeneity within the soil. This related to work by Whitehead (1977) in which no significant difference was found for PCN vertical distribution in the soil. The size of the infestation was determined to allow extensive sampling in all horizontal directions within the area of the cultivation. The number of granules applied needed to be at a high level to allow them, after dilution with the surrounding soil, to be recovered. The location where the microgranules were added varied between the experimental sub-divisions to ensure that any potential microgranule movement remained within the plot. The location of the microgranules within the plots was marked using measuring tapes and fixed markers outside the experimental area. The position of application point was then re-established after cultivation.

5.6.2.2 Individual potato crop cultivations

The individual potato cultivation operations investigated were bed-forming, bed-tilling, de-stoning, planting and harvesting and the machines were configured as for a commercial crop (Table 5.3). The bed-former pulled up beds of 0.6 m in height and 1.83 m width.

The bed-tiller, de-stoner and harvester operate on all the soil in the bed which can result in changes in the height of the bed for this reason their cultivation depths are termed bed depth in Table 5.3.

Table 5.3 Potato cultivation operation specifications.

Cultivator	Cultivator make (model)	Cultivation Depth	Cultivation width (m)	Cultivation speed (km hr^{-1})	p.t.o. for cultivation (rpm)
Bed-former	Dowdeswell (double ridging bodies)	30 cm	3.66	4	-
Bed-tiller	Dowdeswell (powavator fitted with ridging bodies)	Bed depth	1.83	1.5	1000
Destoner	Grimme (colt separator fitted with bed roller)	Bed depth	1.83	1.1	540
Planter	Standen (35 2 row planter)	20 cm	1.83	2.5	-
Harvester	Grimme (Continental 89, with diablo rollers)	Bed depth	1.83	1.5	540

A semi randomised block design was used (Figure 5.10). Each plot was two beds wide (one bed = 1.83 m) and 20 m in length. The plots needed to be two beds wide due to the bed-former forming one complete bed each pass with half beds either side. The microgranules infestations were applied to the top beds (Figure 5.11). For the bed-forming plots, the middle of where the bed was going to be formed was determined, with the application points placed in the middle prior to cultivation. All cultivations were carried out for the plots with the application added prior to their cultivation.

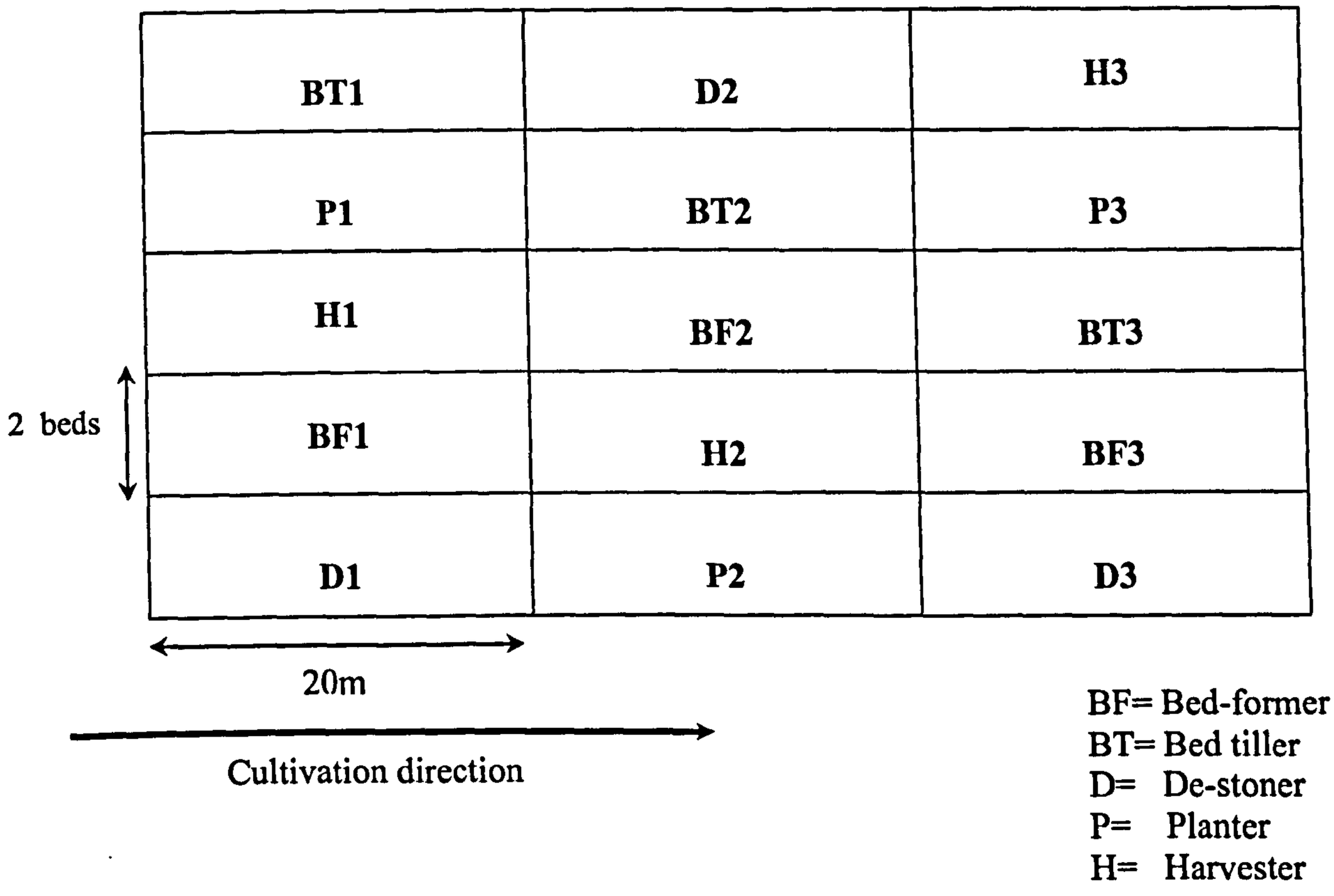


Figure 5.10 Experimental design for individual potato cultivations.

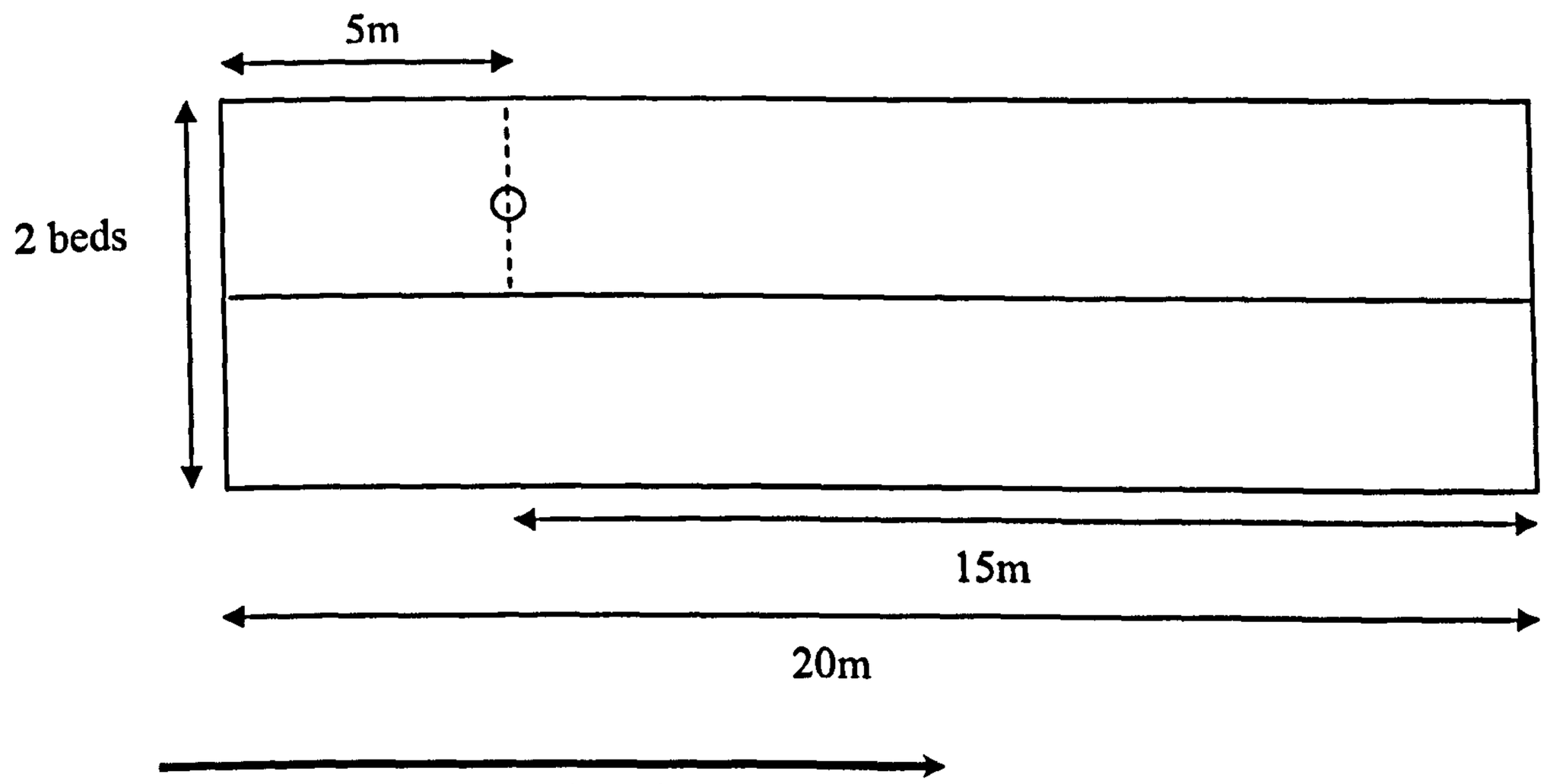


Figure 5.11 Plot design for the individual potato cultivations.

The plots were sampled using a cheese corer (30 cm depth by 4 cm diameter). Cores were taken to the depth of the cultivation machinery operated. Cores were collected at regular

intervals of 0.25 m from the outside the foci point, using the main compass points for direction (N, S, E, W, NE, NW, SE and SW) (Figure 5.12). The South to North direction being that of the cultivation direction. The distances sampled in a given direction corresponded to the expected movement of the microgranules, with the North direction sampled up to 5m, South 2m, and the other directions to 1 m. A metal ring the size of the application point was constructed with 0.25 m bars welded from it at eight 45^{o} intervals this was placed over the application point and fixed in position in line with the cultivation direction. All sampling after cultivation was carried out using this method, except if stated otherwise.

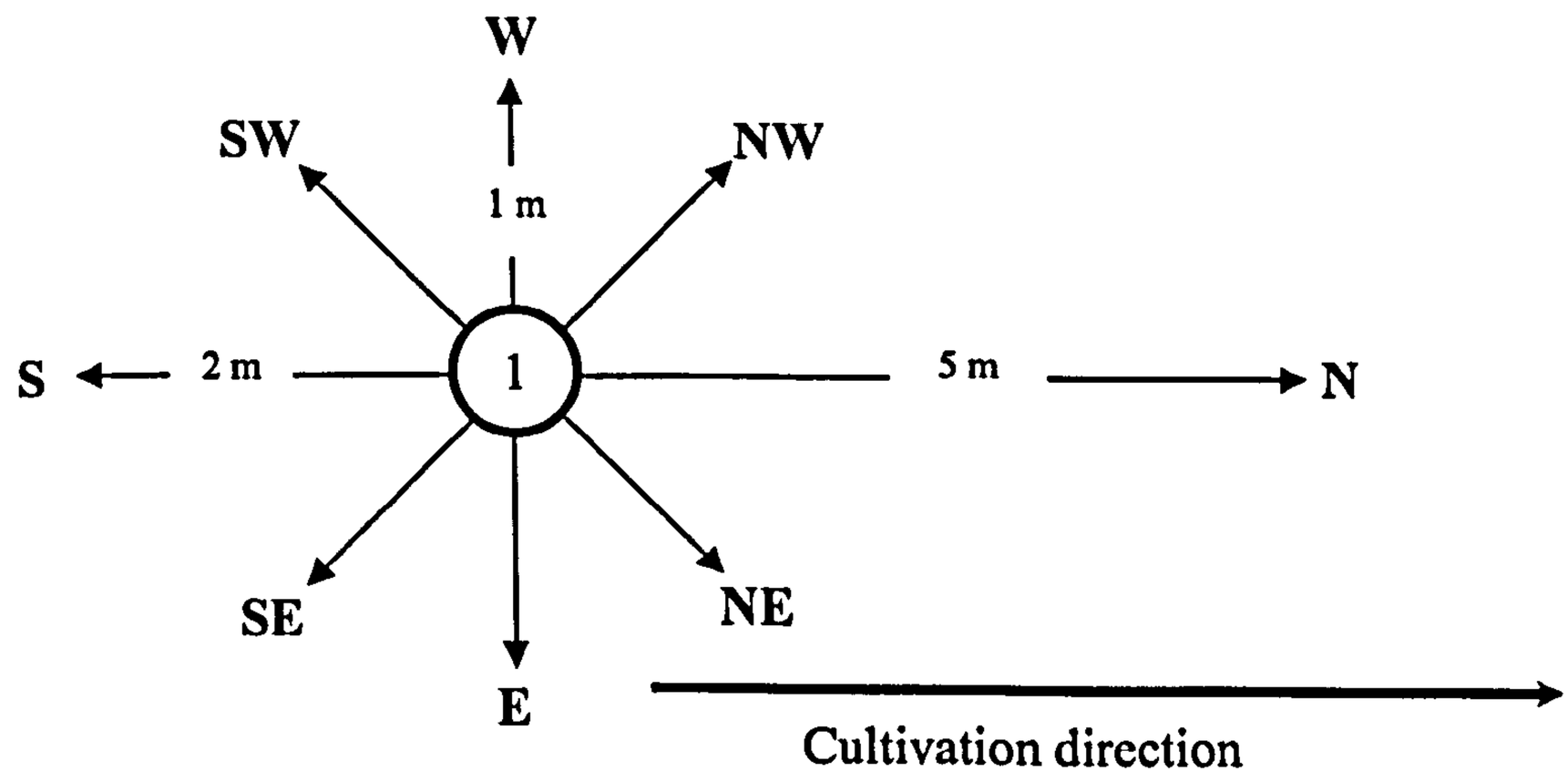


Figure 5.12 Sampling directions within the single potato cultivation plots. 1= Application point.

5.6.2.3 Potato cultivations sequence

The potato sequence experimental design consisted of three replicates of two beds, 30 m in length (Figure 5.13). The microgranules were added in the middle of the plot where the first bed would be formed 10 m into the plot (Figure 5.14). The microgranules were put in prior to the bed-former operation.

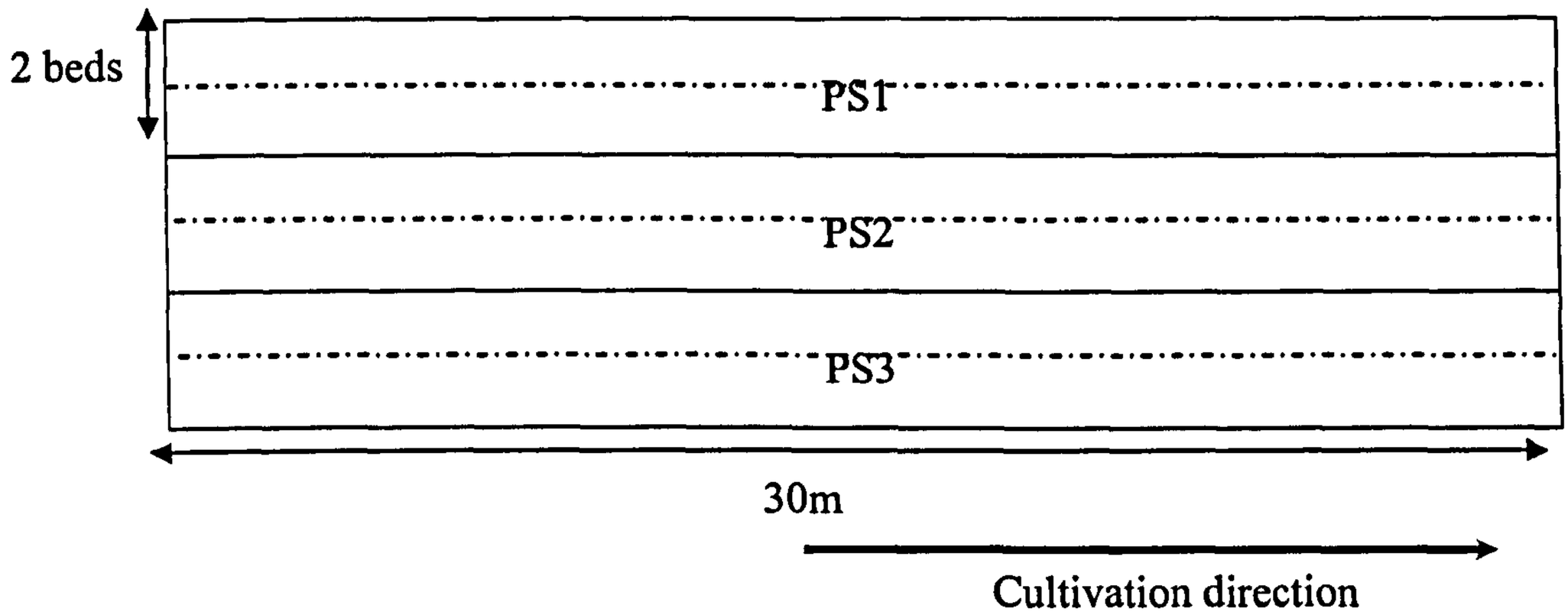


Figure 5.13 Experimental design for potato sequence cultivations.

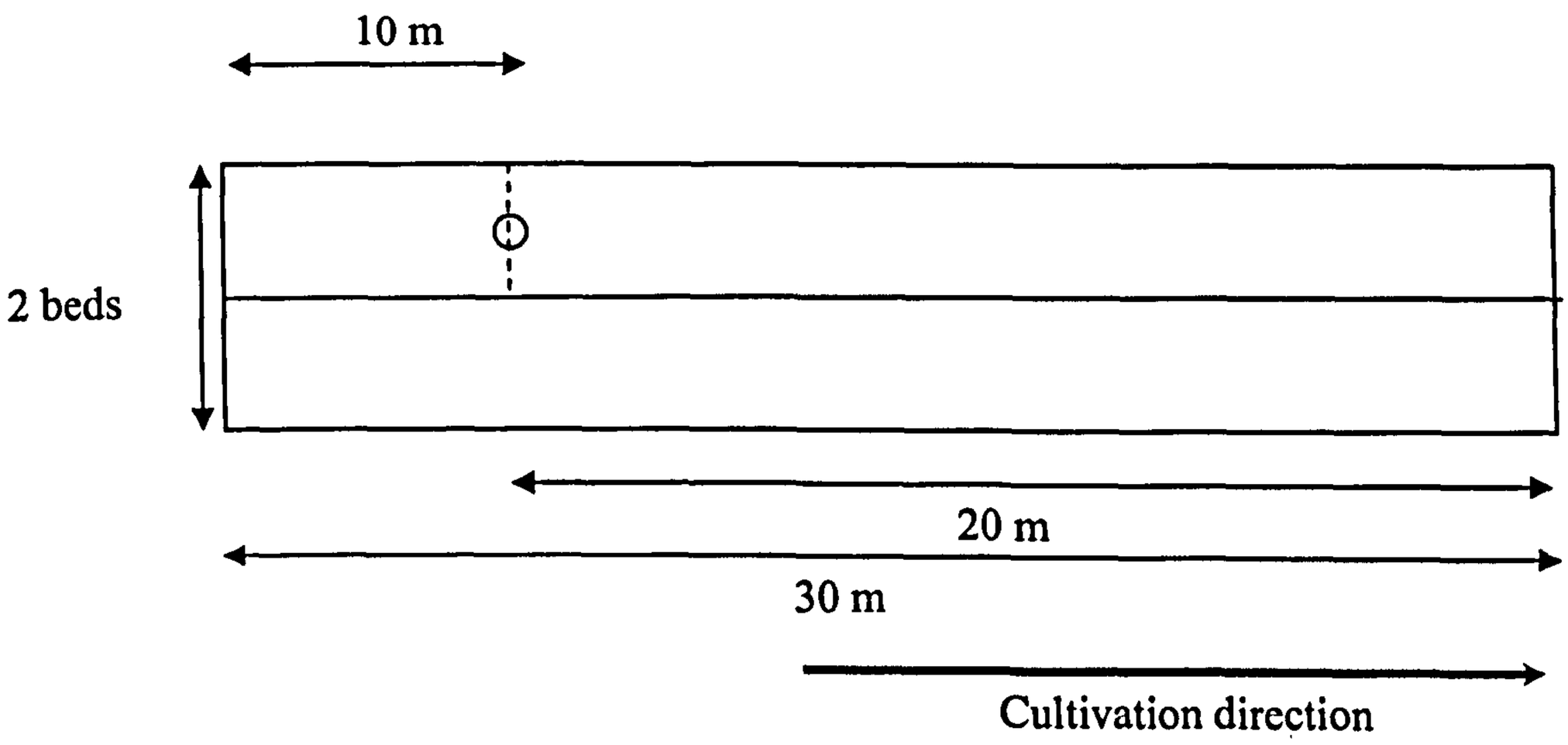


Figure 5.14 Potato cultivations sequence experimental plot design.

The potato sequence plots had all the potato cultivations carried out in sequence; bed-forming, bed tilling, destoning, planting and harvesting. The machines were set up as in the individual potato cultivation experiment. The beds were sampled post potato harvester cultivation. The distances of sampling were North 15 m, South 5 m and 1 m in the other directions, at 0.25 cm intervals.

5.6.2.4 Bed flattening cultivations

After harvest the beds, although partially flattened by the harvesting operation, need to be flattened prior to cereal cultivations being implemented. The cultivation machinery used for this operation is dependent on the available machinery to the grower. Three pieces of cultivation equipment that can be used are a spring tine, sub-soiler and terra disc (Table 5.4).

The experimental area underwent all the cultivations in a potato crop prior to being set up for the bed flattening operations. The experimental design was a semi randomised block design. Each plot was 30 m in length to allow the tractor to reach its required speed within the plot (Figure 5.15). Each plot was one bed wide with the microgranules added in the middle of the bed 15 m along the plot (Figure 5.16).

Table 5.4 Bed flattening cultivation operation specifications.

Cultivator	Cultivator make (model)	Cultivation depth (cm)	Cultivation width (m)	Cultivation speed (km/ hr^{-1})
Spring tine	Kverneland (Kultisvans, three rows of 9 tines)	15	3	10
Terra-disc	Lemken (Smagagd 8)	15	3	10
Sub-soiler	M^{c}Connell (5 leg with crumbler roller attached)	40	3	6

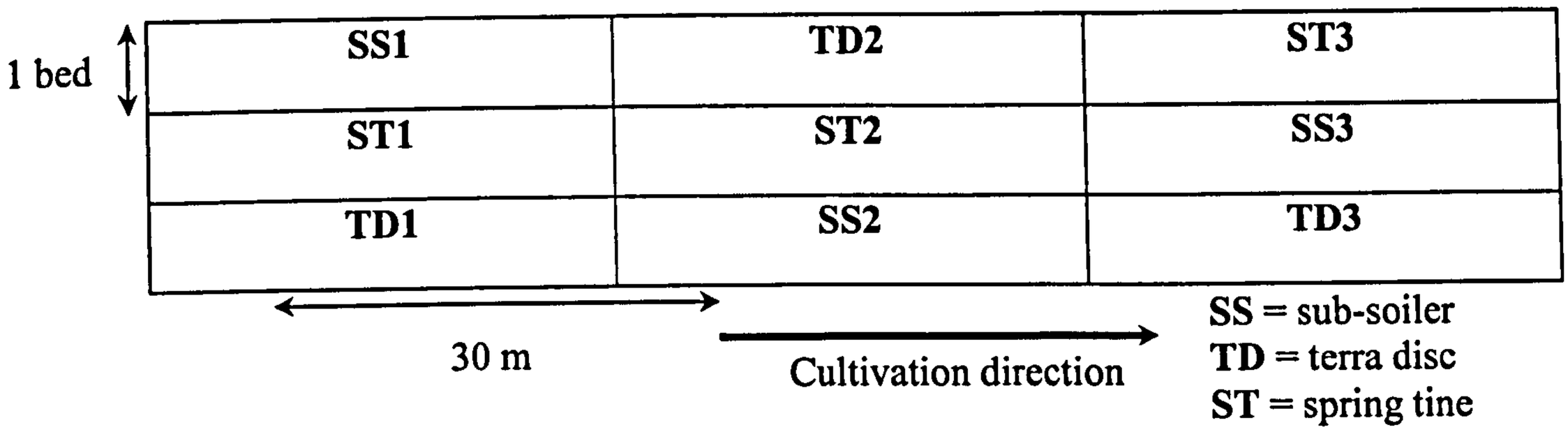


Figure 5.15 Experimental design for bed flattening operations.

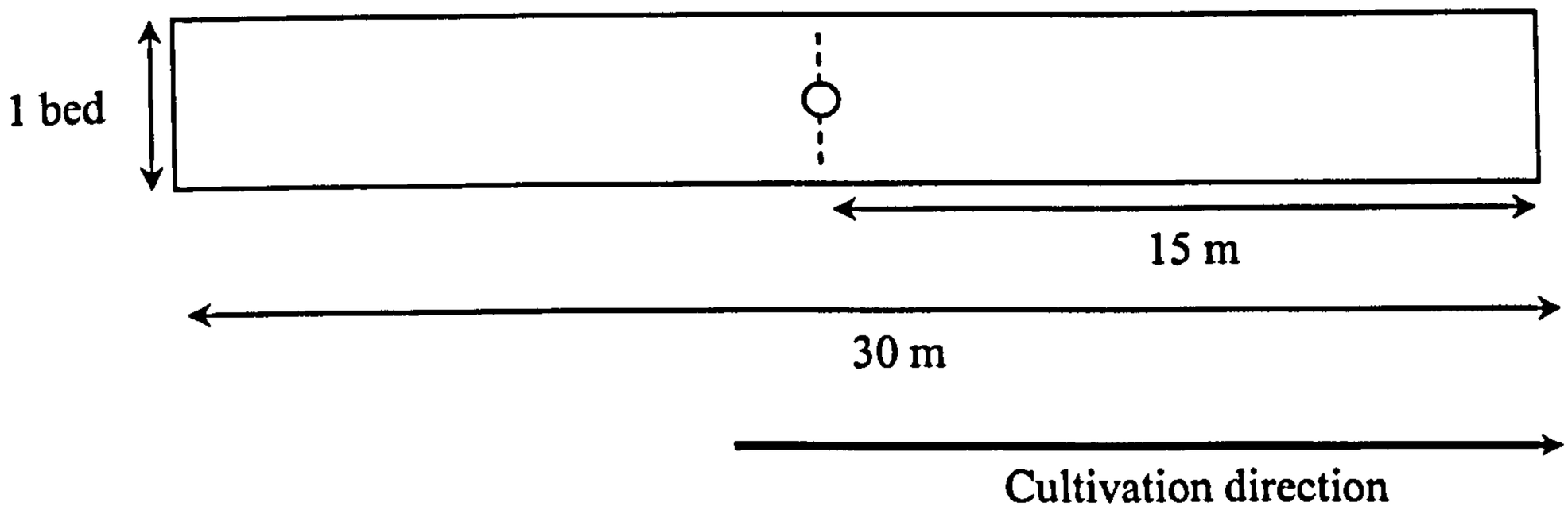


Figure 5.16 Experimental plot design for potato bed flattening cultivations.

5.6.2.5 Plough cultivations

Both chisel plough and mouldboard plough were operated at standard speeds and depths (Table 5.5). The experimental design was a semi randomised block design. The plots were 30 m in length and 3 m wide (Figure 5.17). The experimental site had not undergone the potato cultivations prior to the experiment. The microgranules were added 15 m along the plot in the middle (Figure 5.18).

Table 5.5 Plough cultivation

Cultivator	Cultivator make (model)	Cultivation depth(cm)	Cultivation width (m)	Cultivation speed (km/
Mouldboard plough	Dowdeswell (DP 100S 4 furrow plough)	20	3	7
Chisel plough	Generic Chisel plough 7 fixed tines	20	3	9

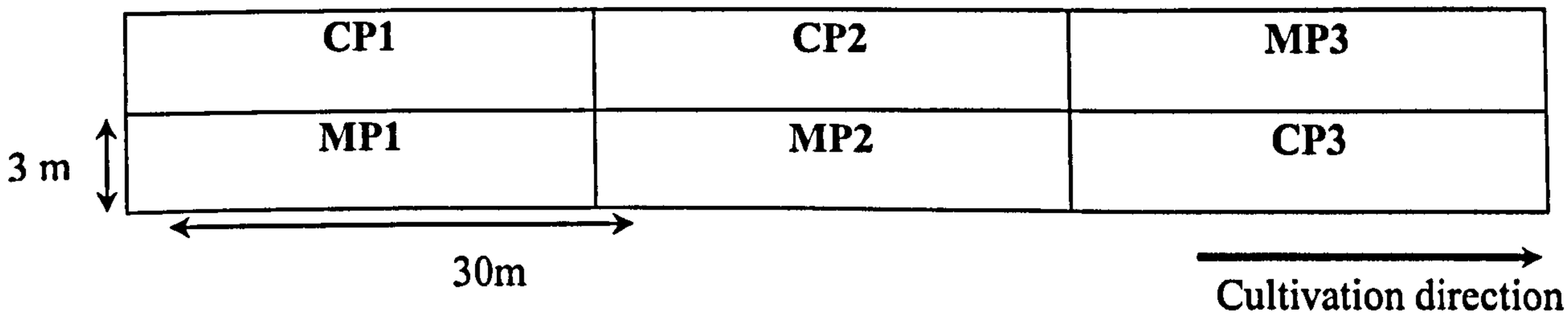


Figure 5.17 Experimental design for plough cultivations

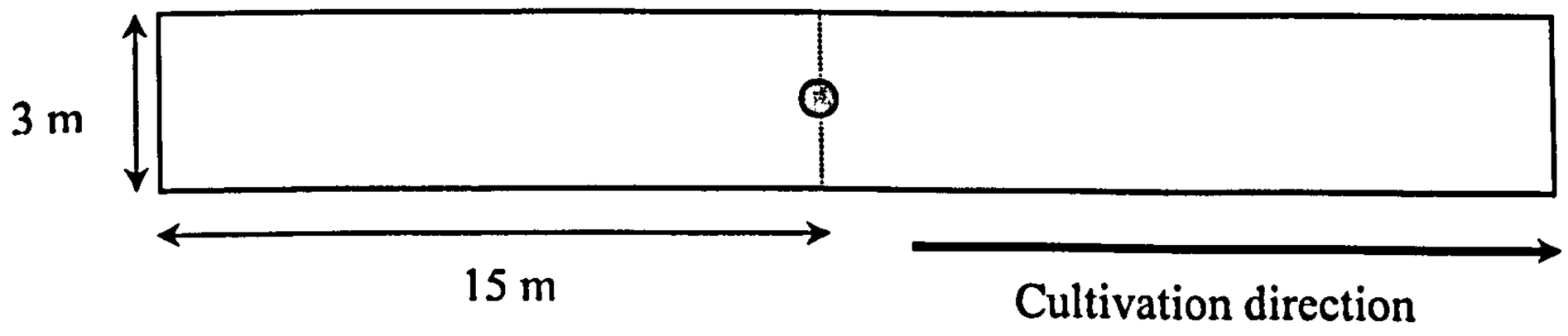


Figure 5.18 Experimental plot design for plough cultivations

5.6.2.6 Individual Cereal cultivations

Two secondary cultivation operations commonly used for seedbed preparation were investigated, the power harrow and spring tine. These were configured and operated as for commercial field operations (Table 5.6) The experimental design was a semi randomised block (Figure 5.19). Plot size was 10 m long by 3 m wide. The experimental area had undergone all the cultivations involved in a potato crop and bed flattening (using terra-

disc) prior to the experiment. The spring tine operation was used on all the plots followed by the power harrow. The foci were added 3 m into the plots in the middle prior to their cultivation operation (Figure 5.20). The cultivations were carried out in the sequence of spring tine then power harrow. Post cultivation the plots were sampled as for the individual potato cultivation experiment.

Table 5.6 Cereal cultivation operation specifications

Cultivator	Cultivator make (model	Cultivation depth (cm)	Cultivation width (m)	Cultivation speed (km/ hr^{-1})	p.t.o. for cultivation (rpm)
Spring tine	Kvernelands (Kultisvans, three rows of 9 tines)	15	3	10	-
Power harrow	Dowdeswell (PH 300)	15	3	6	540

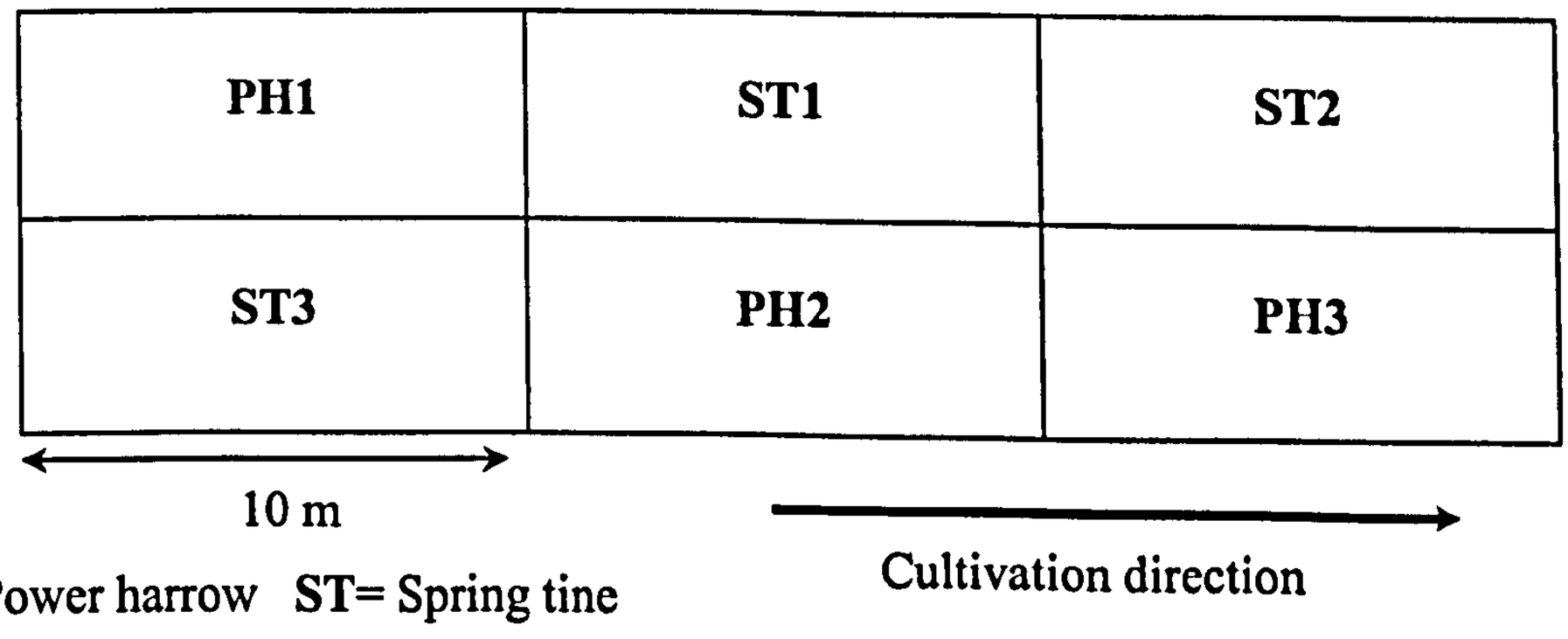


Figure 5.19 Experimental design for individual cereal cultivation operations

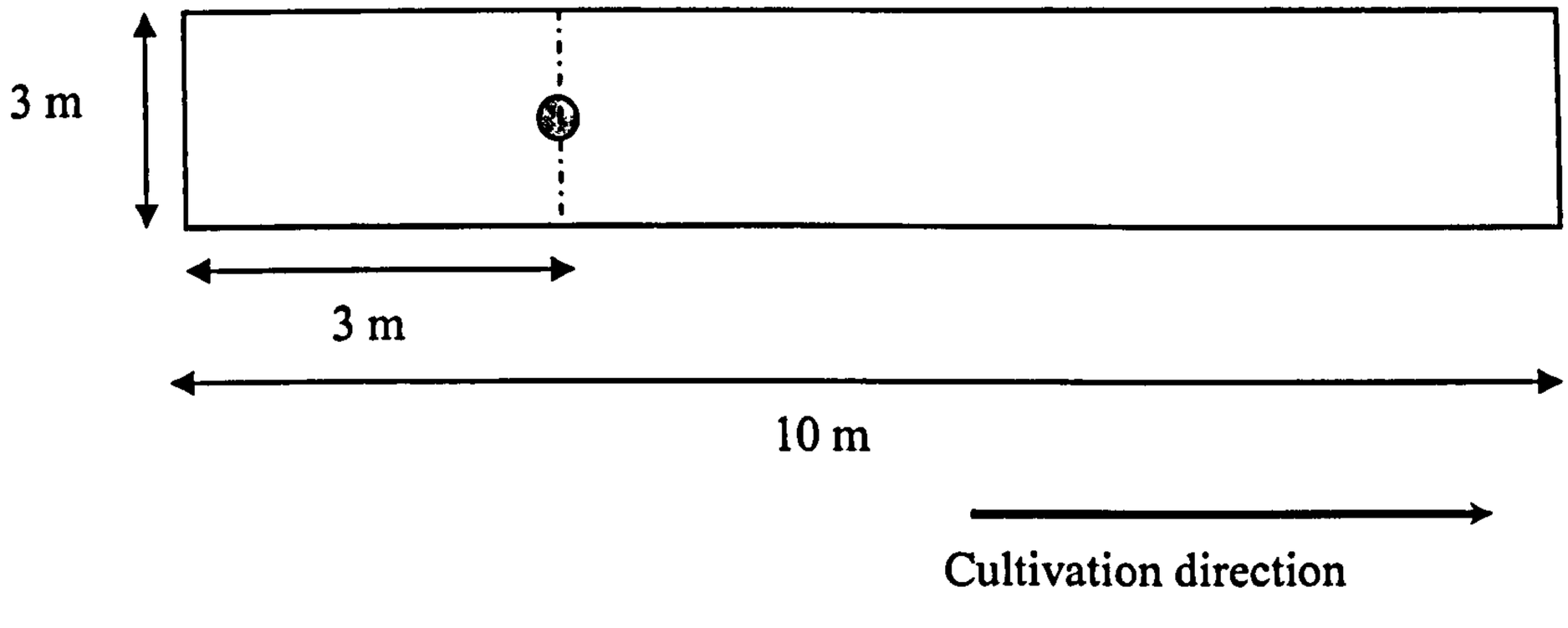


Figure 5.20 Experimental plot design for individual cereal cultivation operations

5.6.2.7 Winter wheat cultivation sequence

The winter wheat cultivation sequence comprised the operations usually carried out after a potato crop. This would normally take place in the autumn. The winter wheat sequence consisted of bed flattening then spring tines and power harrowing. The experiment consisted of three replicate plots of ten potato beds in length and 10 m wide (Figure 5.21). The potato beds had undergone all the potato cultivations prior to the setting up of the plots.

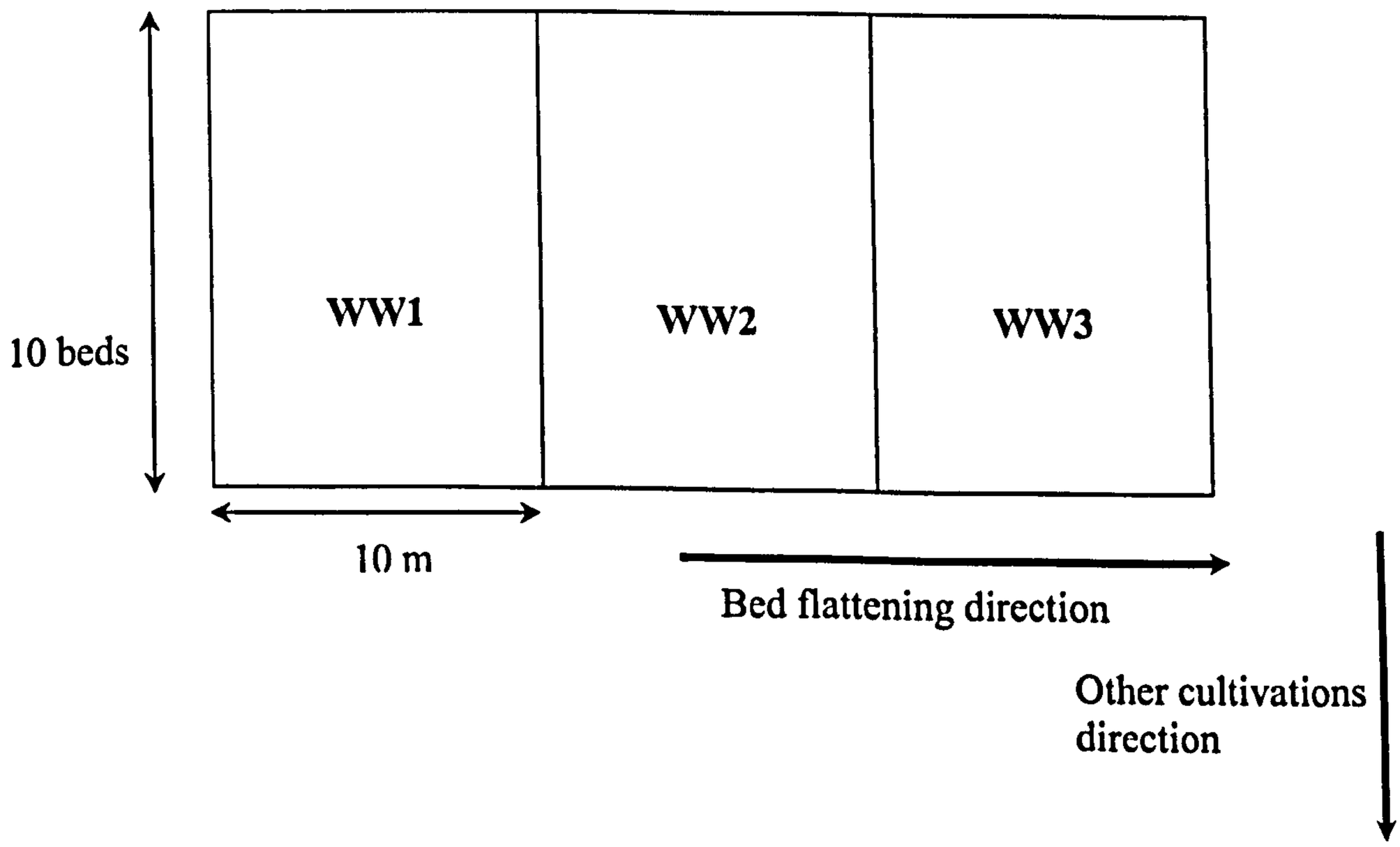


Figure 5.21 Experimental design for winter wheat cultivation sequence

The microgranules were put in the middle of the fourth bed, three metres from the left side, to take into account the two different directions of cultivation within the experiment. Bed flattening (using terra-disc) along the beds and the cereal cultivations were carried out perpendicular to the beds (Figure 5.22). The cultivation machinery was configured and operated as for the bed flattening experiment and the individual cereal cultivations experiment.

The sampling was carried out after power harrowing; to take into account the two different directions of cultivation undergone the sampling regime was altered, with N being the direction that the cereal cultivation were carried out towards (Figure 5.22).

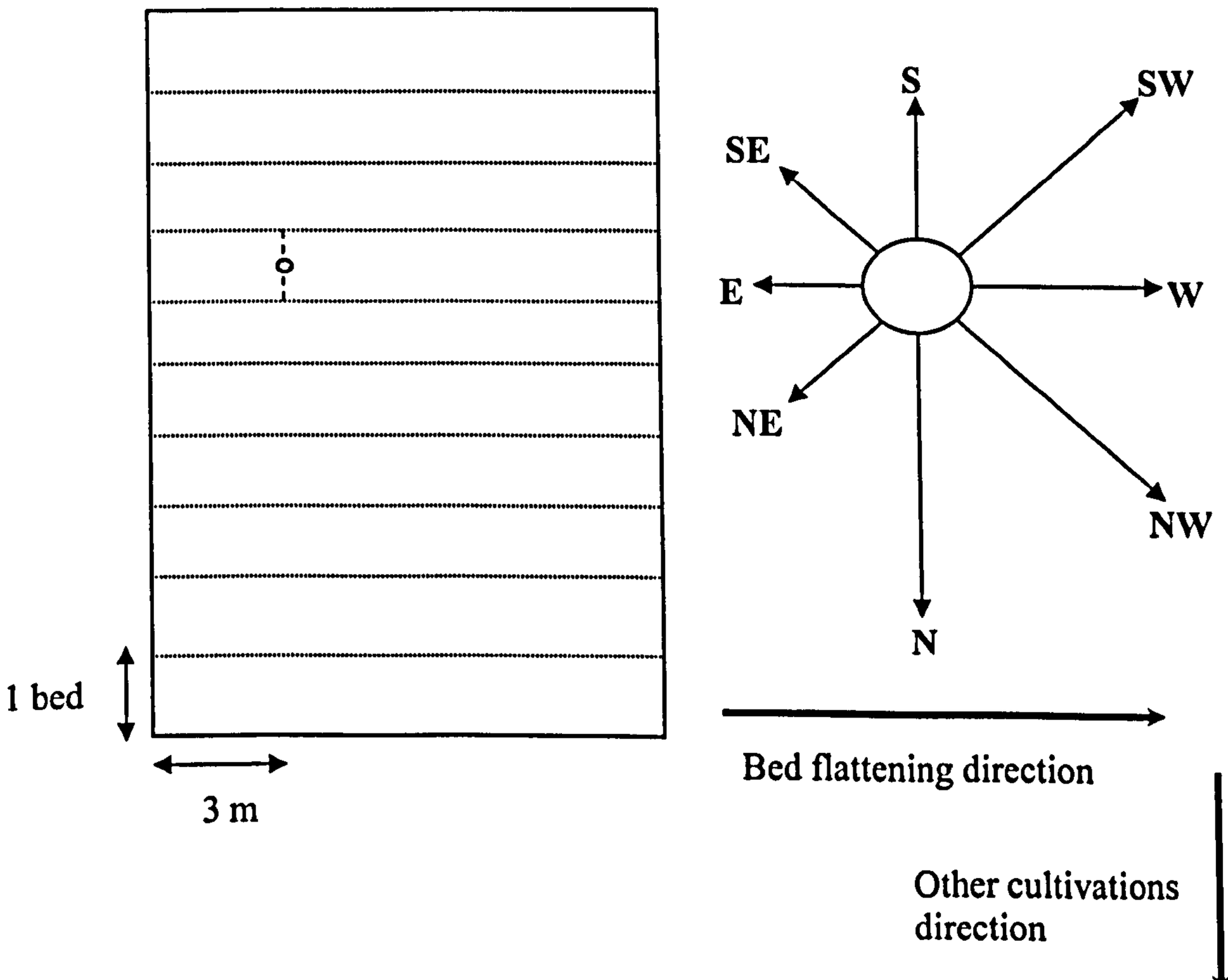


Figure 5.22 Plot design and sampling methodology (sampling distance= N 10 m, NE 2 m, E 2 m, SE 2 m, S 4 m, SW 5 m, W 5 m and NW 5 m) for winter wheat sequence cultivations.

5.6.2.8 Spring barley cultivation sequence

The spring barley cultivation sequence experimental area had been subjected to all the potato cultivations. This experiment consisted of those cultivations carried out after a potato crop to prepare the field for spring barley. This was the same as that of the winter wheat except with the addition of ploughing prior to the cereal cultivations. The cultivation sequence was bed flattening, ploughing, spring tines and then power harrowing. The ploughing operation would normally take place after winter having been bed flattened in the autumn. The mouldboard plough was used and set for cereal cultivations of 20 cm depth. The plots were 12 beds in length and 10 m wide (Figure 5.23). The microgranules were put in the middle of the fifth bed 3 m in from the left side of the plot (Figure 5.24). The sampling was carried out the same as that for the winter wheat sequence experiment, except for N and S these were sampled to 12 m and 6 m respectively.

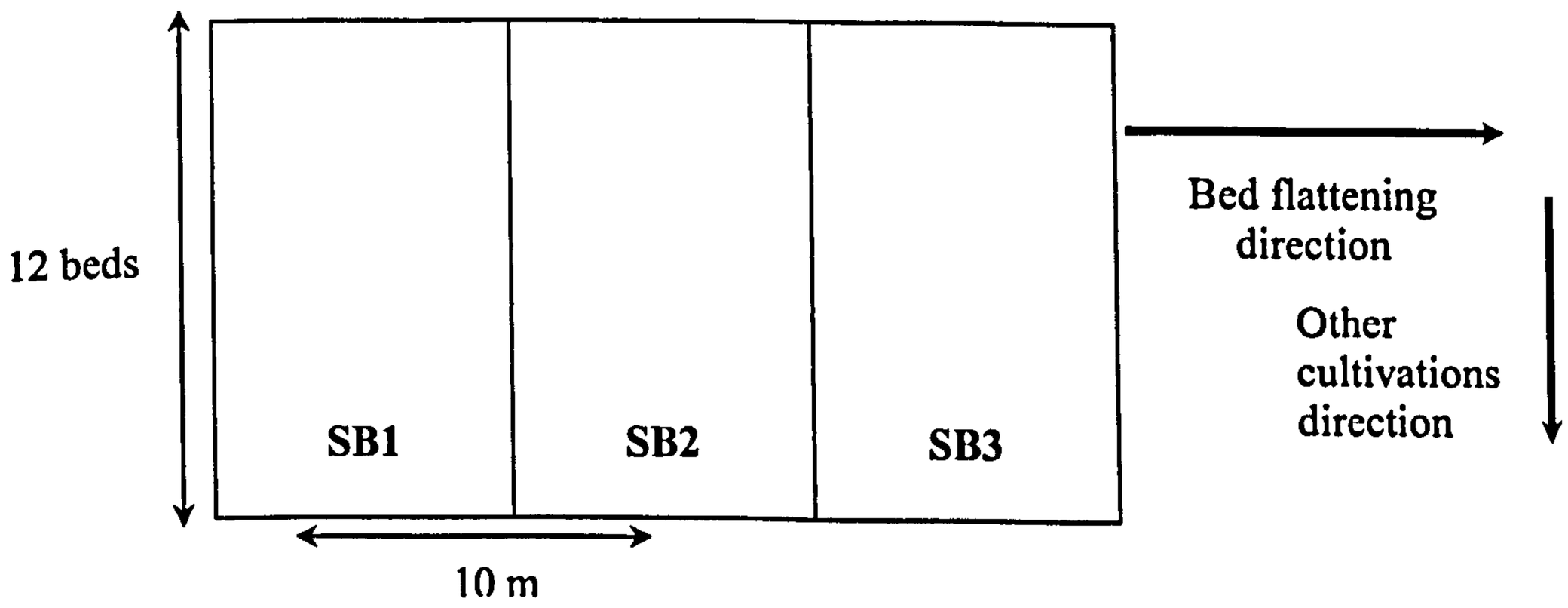


Figure 5.23 Experimental design, for spring barley cultivation sequence.

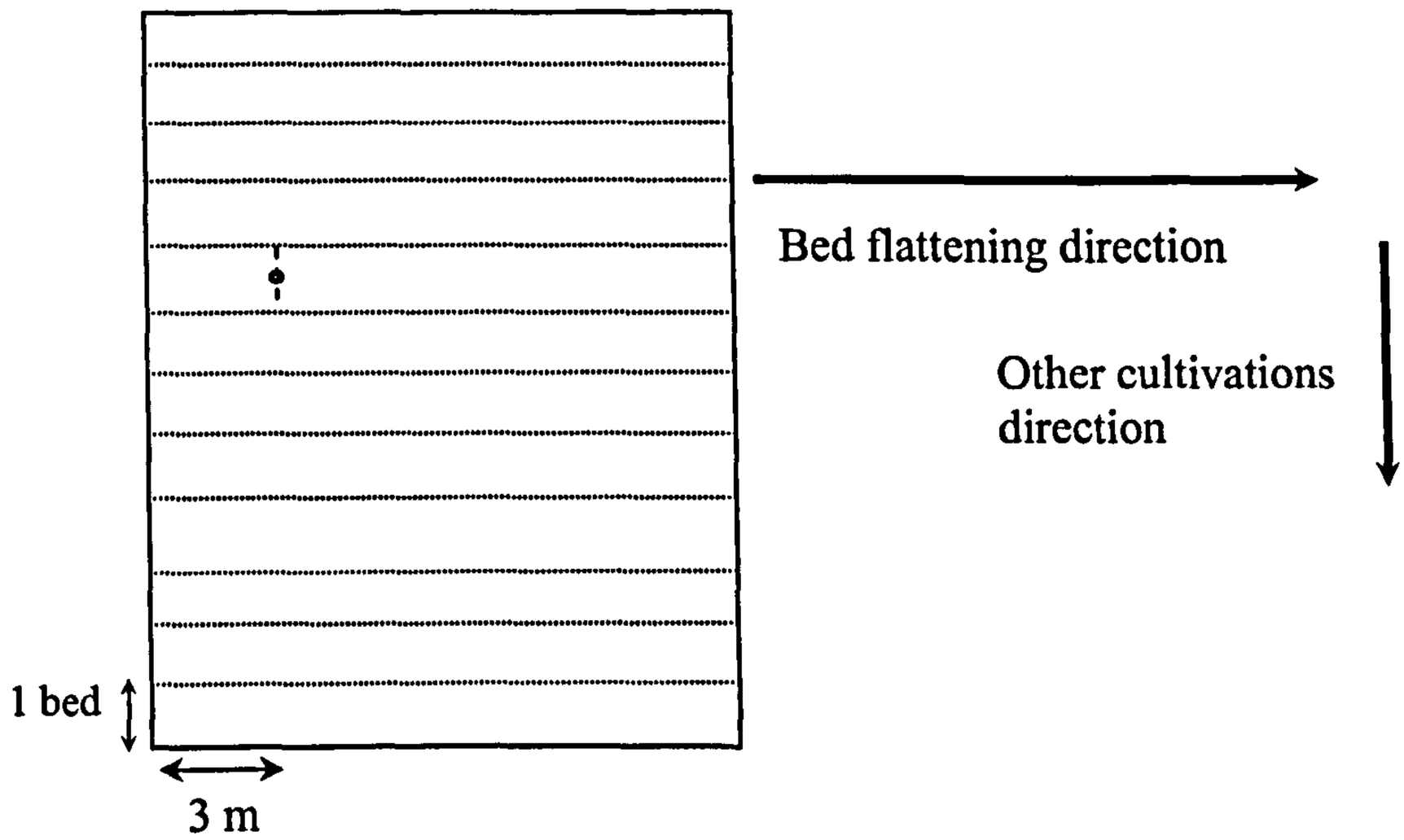


Figure 5.24 Plot design for spring barley sequence cultivations.

5.6.2.9 Sample processing

The soil samples were put in an oven at 80^0C for 5 days and were then coarsely sieved (4 mm aperture) and weighed.

The samples were assessed in a dark room using a Ultra-Violet (UV) paddle as the light source. A sample was poured onto a tray (1 m^2) and any small clods of soil were gently crushed (Plate 5.3). The tray was divided into 9 sub-sections. The granules present showed up as bright pink under UV light (Plate 5.4), and were counted.

Plate 5.3 Sample processing set up.

The samples were processed out from the application point sample in the eight directions, with all samples for the 6 lateral directions and South processed. The samples in the North direction, for the individual cultivations, were processed up to 3 m. Thereafter, if 3 consecutive samples did not contain any granules the remaining samples were not processed. For the cultivation sequences experiments the distance of samples processed in the North direction was 5 m before this rule was adopted.

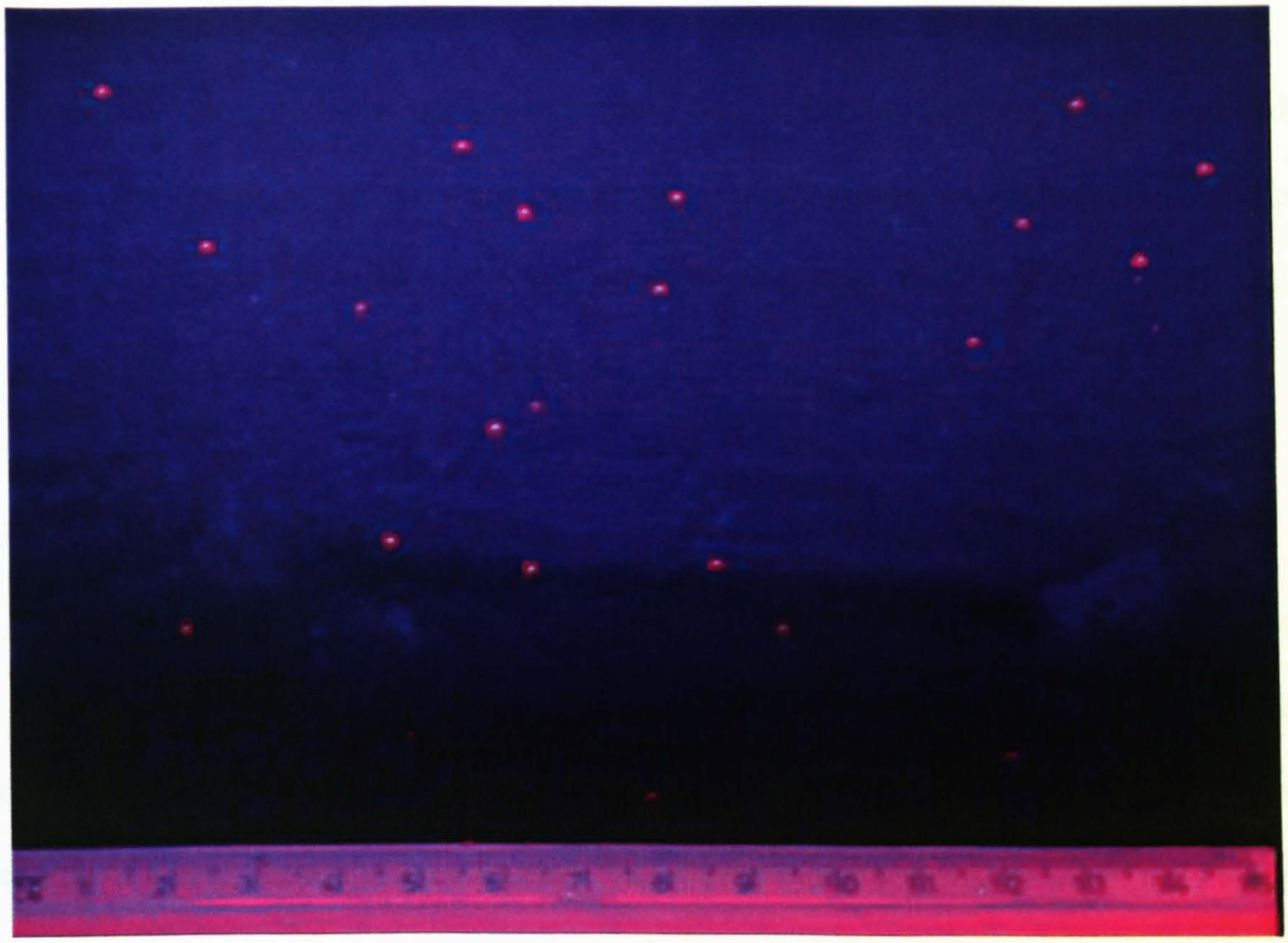


Plate 5.4 Soil sample under UV light.

5.6.2. Data analysis

Data from the experiment was analysed for the individual cultivations in the direction of cultivation and lateral movement using ANOVA. The assumption that the microgranules act independently of each other was taken, so that the range of movement by the cultivation operation was analysed. All analysis was carried out using Minitab 12 (MINITAB INC.)

5.6.3 Results and discussion

The movement of the microgranules by individual cultivations are presented, discussed and compared. The results of the sequence experiments are discussed and compared with the potential cumulative effects from the individual cultivations. The implications for cyst movement within a crop rotation are discussed.

5.6.3.1 Potato cultivations

Bed former. No movement of the microgranules from the application point was found after this cultivation operation. This was probably due to the application point being in the middle of the plots. After ploughing the bed former lifts the soil using the ridging bodies either side of where the bed is to be formed. The movement of soil results in the beds being approximately double that of the plough depth. Due to the application point being at a depth of 30 cm prior to the operation, no soil from the depth of the application was sampled (Figure 5.25). Although no movement was found for this machinery it is an intensive cultivation operation, by setting the application point off-centre of where the bed was to be formed it is likely that considerable movement would have resulted.

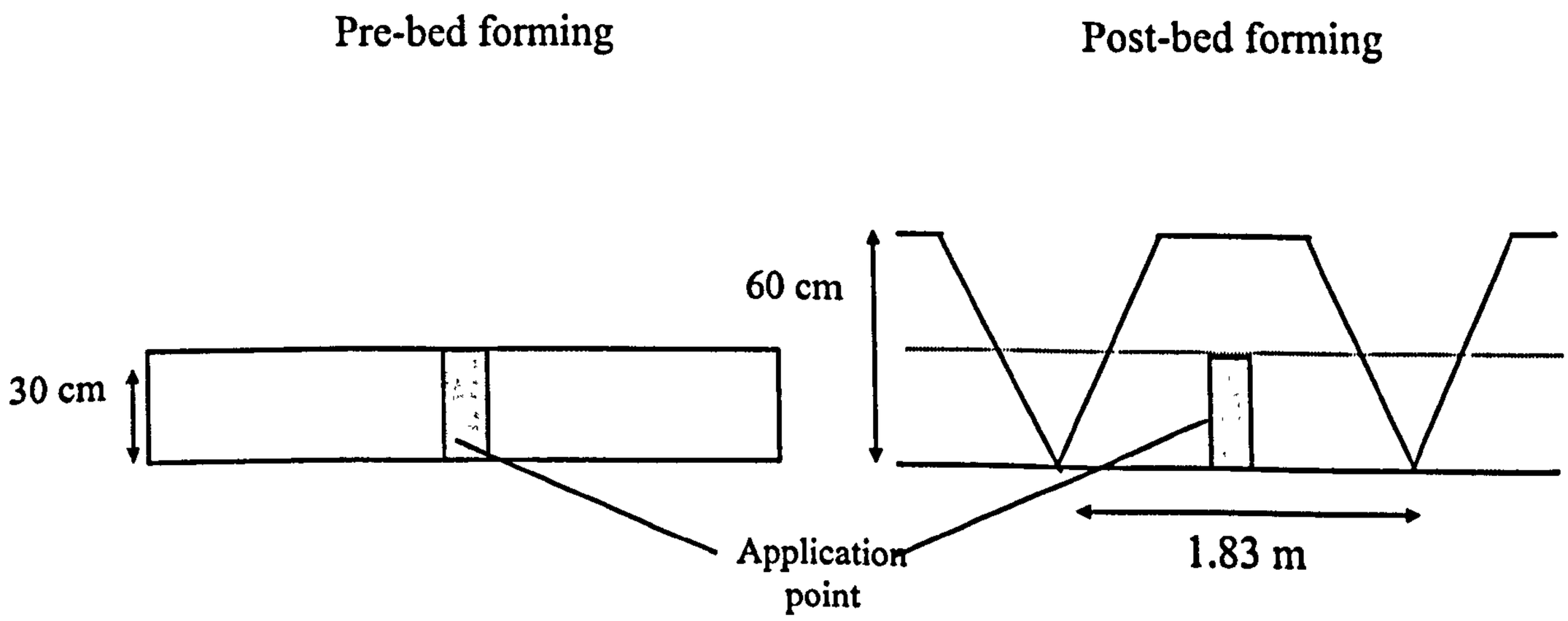


Figure 5.25 Diagrammatic cross section of pre- and post- bed former plots.

Bed tiller. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.7. Over 98% of the total number of microgranules were found along the axis of cultivation, the majority were in the cores less than 1 m behind the application point. S 0.25 m had the most microgranules; the numbers in the cores relative to this point showed a declining trend. Microgranules were moved up to 3.25 m beyond the application point and up to 0.5 m before (Figure 5.26).

The microgranules were almost evenly distributed before and after the application point, along the axis S to N axis. The bed tiller spikes are fixed on a rotor, powered by the tractor engine. The limited movement behind the application point is likely to be the result of the limited period of contact that these microgranules have with the spikes as they were moved behind the bed tiller. The microgranules found past the application point, although similar in number with those prior to the application, were found to have been moved considerably further. Microgranules were found in a decreasing trend up to 3.25 m from the application point. The reason for this is probably due to repeated contact with the spikes. The decreasing trend away from the application is likely to be due to the microgranules at each point being potentially unmoved, moved back, moved laterally or forward.

Little lateral movement was found; NE had microgranules moved up to 0.75 m and in the E direction microgranules were found at 0.25 m (Table 5.8). Lateral movement is to be expected as the bed tiller is designed to break up clods in a location. This requires the beds to be re-formed by the fitted ridging bodies on the back of the bed tiller. This re-forming potentially could have moved the microgranules back into the centre of the beds, resulting in little lateral movement.

Table 5.7 Percentage of total microgranules found in the directions and at the distances of sampling, for the bed tiller operation.

Cultivation Direction ↑	Distance from application point (m)				
	< 1 m	1 – 2 m	2 – 3 m	3 – 4 m	4 – 5 m
N ↑	23.8	15.5	8.4	0.6	0
NE ↗	1.2				
E →	0.1				
SE ↘	0				
S ↓	50.4	0			
SW ↙	0				
W ←	0				
NW ↖	0				

Table 5.8 Mean number of microgranules (SE) moved laterally to cultivation direction by bed tiller operation.

Cultivation Direction ↑	Distance from the application point (m)			
	0.25	0.50	0.75	1.0
NE ↗	3.6 (2.3)	1.2 (0.4)	0.4 (0.4)	0
E →	0.3 (0.3)	0	0	0

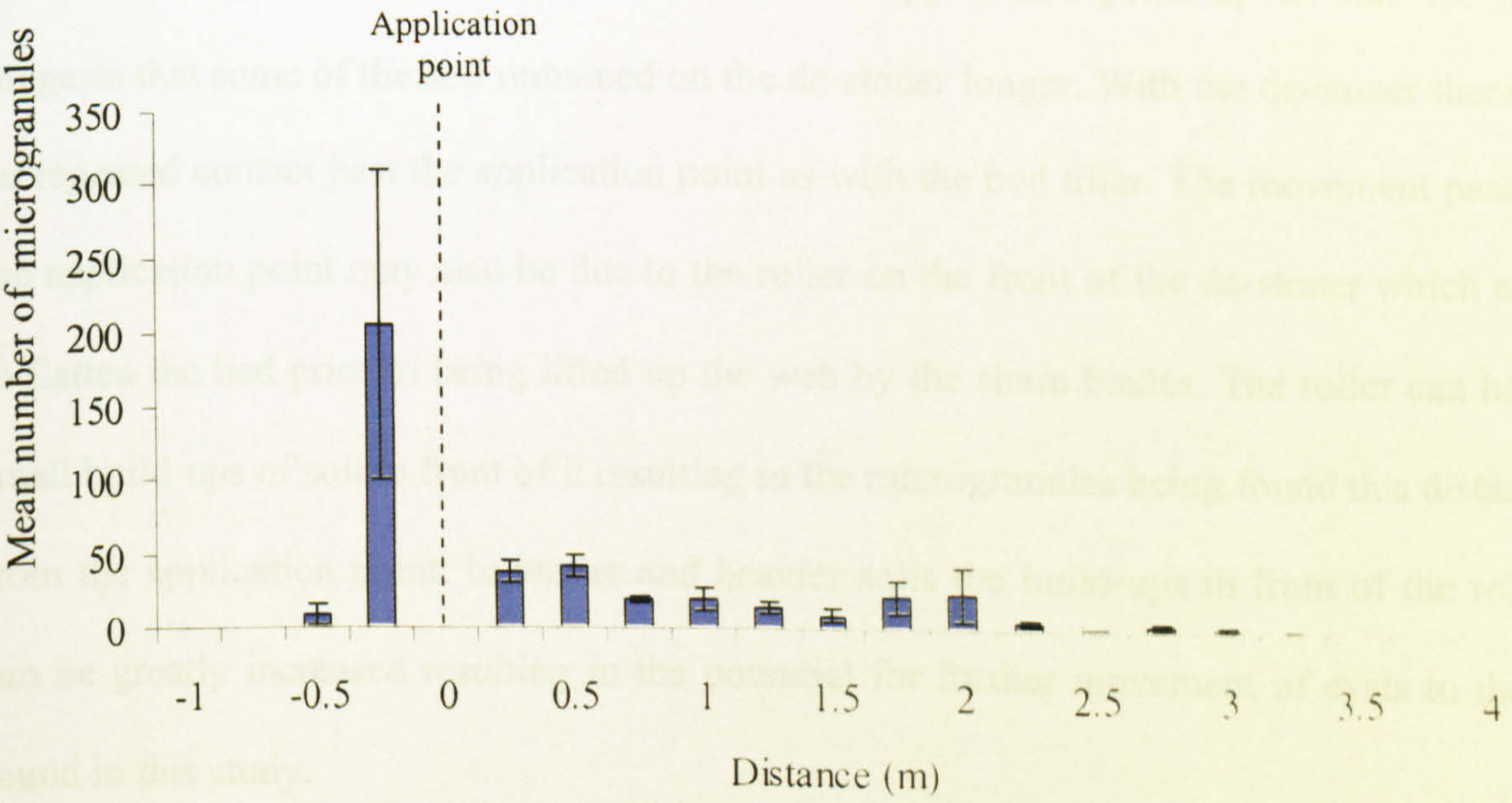


Figure 5.26 Mean number of microgranules in the S to N direction for the bed tiller operation (with +/- SE bars)

De-stoner. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.9. Over 98% of the total number of microgranules recovered were found along the axis of cultivation, the majority being found in the cores less than 1 m North (62%). The distance with the most microgranules was N 0.5 m; the number of microgranules recovered in the cores relative to this showed a declining trend in both S and N directions. Microgranules were found up to N 4.25 m and up to S 1.25 m (Figure 5.27).

The action of the de-stoner is to slice into the bed and lift the bed up and along a web. Soil falls through the web but any stones or clods are taken up the bed and dropped into a windrow. At 0.75 m past the application point the highest concentration of microgranules was found, showing that the highest proportion of soil from the application point dropped through the web at this point. The speed of the tractor relative to the de-stoner web is important in terms of the distance that the microgranules are moved. Microgranules were found prior to application point up to 1.25 m suggesting that some of the microgranules and soil travelled further up the web before being dropped, relative to the speed of the tractor. Microgranules were also found after the application point up to 4.25 m, this suggests that some of the soil remained on the de-stoner longer. With the de-stoner there is no repeated contact past the application point as with the bed tiller. The movement passed the application point may also be due to the roller on the front of the de-stoner which acts to flatten the bed prior to being lifted up the web by the share blades. The roller can have small build-ups of soil in front of it resulting in the microgranules being found this distance from the application point. In wetter and heavier soils the build-ups in front of the roller can be greatly increased resulting in the potential for further movement of cysts to those found in this study.

Microgranules were found in the three westerly sampling directions with W and SW at 0.25 m, and NW up to 0.75 m (Table 5.10). As with the bed tiller, the de-stoner is meant to

improve the bed without resulting in much lateral movement of soil, although, as with the bed tiller, some lateral movement will take place. On the side that lateral movement was found the cross conveyor was out to drop the stones into the windrow; the extra weight on this side of the cultivator could have resulted in some lateral soil movement, although no leaning of the machine was observed.

One factor not measured in this experiment was the potential for cysts to be dropped in the windrows from the cross conveyor. If the soil is wet more clods and stones covered in mud can be lifted and dropped in the windrows potentially increasing the movement of cysts. These cysts then have the potential to create new foci points in subsequent potato crops.

Table 5.9 Percentage of total microgranules found in the directions and at the distances of sampling, for the de-stoner operation.

Cultivation Direction ↑	Distance from the application point				
	< 1 m	1 – 2 m	2 – 3 m	3 – 4 m	4 – 5 m
N ↑	62.2	13.8	2.3	1.3	0.2
NE ↗	0				
E →	0				
SE ↘	0				
S ↓	4.4	0.1			
SW ↙	0.1				
W ←	1.1				
NW ↖	14.4				

Table 5.10 Mean number of microgranules (SE) moved laterally to cultivation direction by de-stoner operation.

Cultivation Direction ↑	Distance from the application point			
	0.25	0.50	0.75	1.0
NW ↖	76.1 (45.3)	11.6 (7.4)	1.2 (0.7)	0
W ←	7.2 (4.2)	0	0	0
SW ↙	0.7 (0.4)	0	0	0

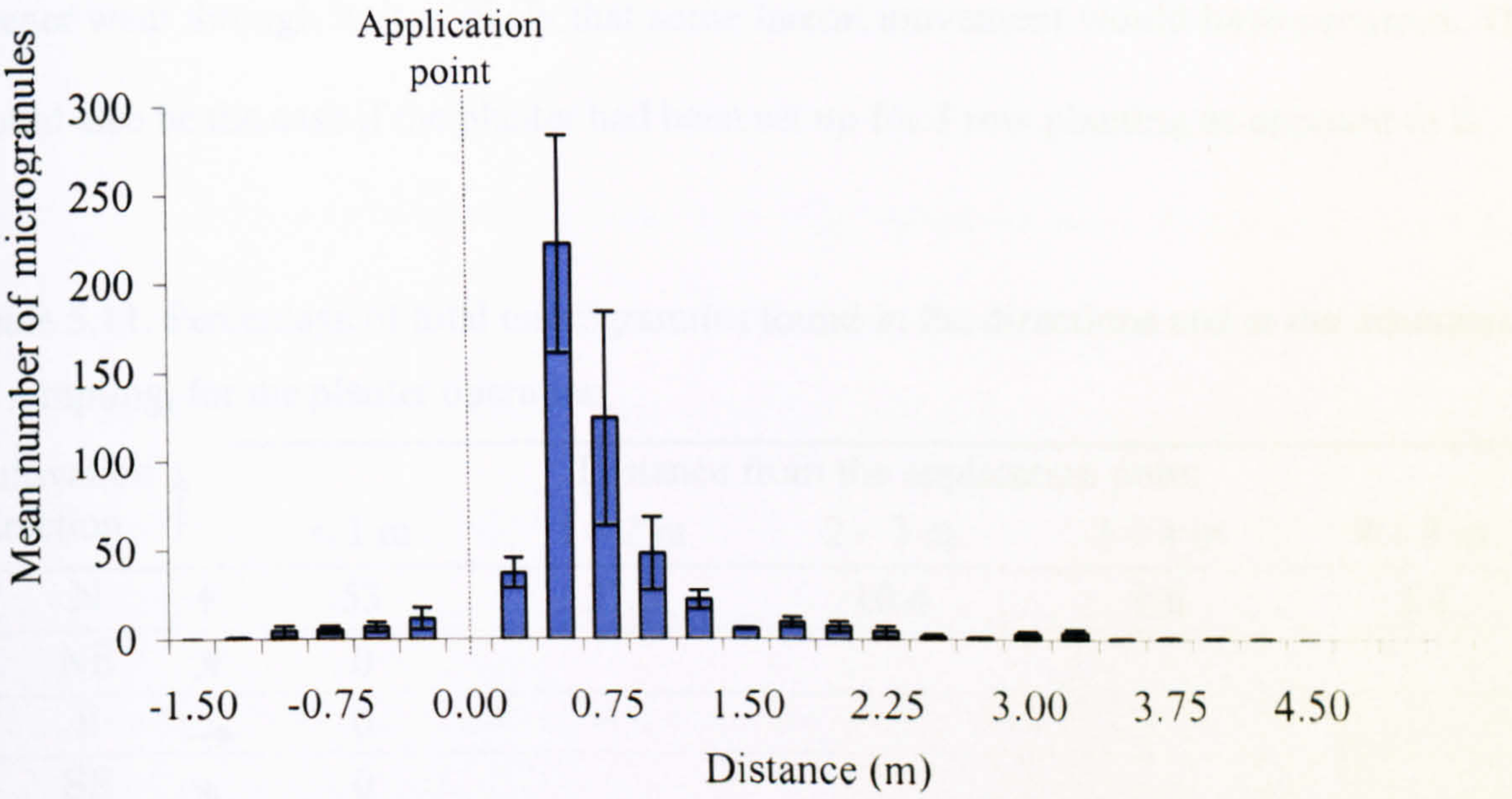


Figure 5.27 Mean number of microgranules in the S to N direction for the de-stoner operation (with +/- SE bars).

Planter. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.11. Microgranules were only found past the application point along the axis of cultivation. Within 1 m of the application point, 53% of the microgranules were recovered. The distance with the most microgranules was 0.5 m N; the number of microgranules recovered in the cores relative to this showed a declining trend. Microgranules were found in cores up to 5 m beyond the application point (Figure 5.28).

The planter has no mechanical cultivation parts; this is the probable reason why no microgranules were found behind the application point. The movement up the bed is probably due to the furrow closing board at the back of the planter dragging soil and microgranules along the bed.

There was no lateral movement for this operation. The furrow openers would have passed either side of the application point, as would the ridging bodies, causing no movement of

the microgranules. If the application point were inserted into the bed off-centre so a furrow opener went through it, it is likely that some lateral movement would have occurred. This would also be the case if the planter had been set up for 3 row planting as opposed to 2.

Table 5.11. Percentage of total microgranules found in the directions and at the distances of sampling, for the planter operation.

Cultivation Direction ↑	Distance from the application point				
	< 1 m	1 – 2 m	2 – 3 m	3 – 4 m	4 – 5 m
N ↑	53	27.5	10.4	5.8	3.3
NE ↗	0				
E →	0				
SE ↘	0				
S ↓	0	0			
SW ↙	0				
W ←	0				
NW ↖	0				

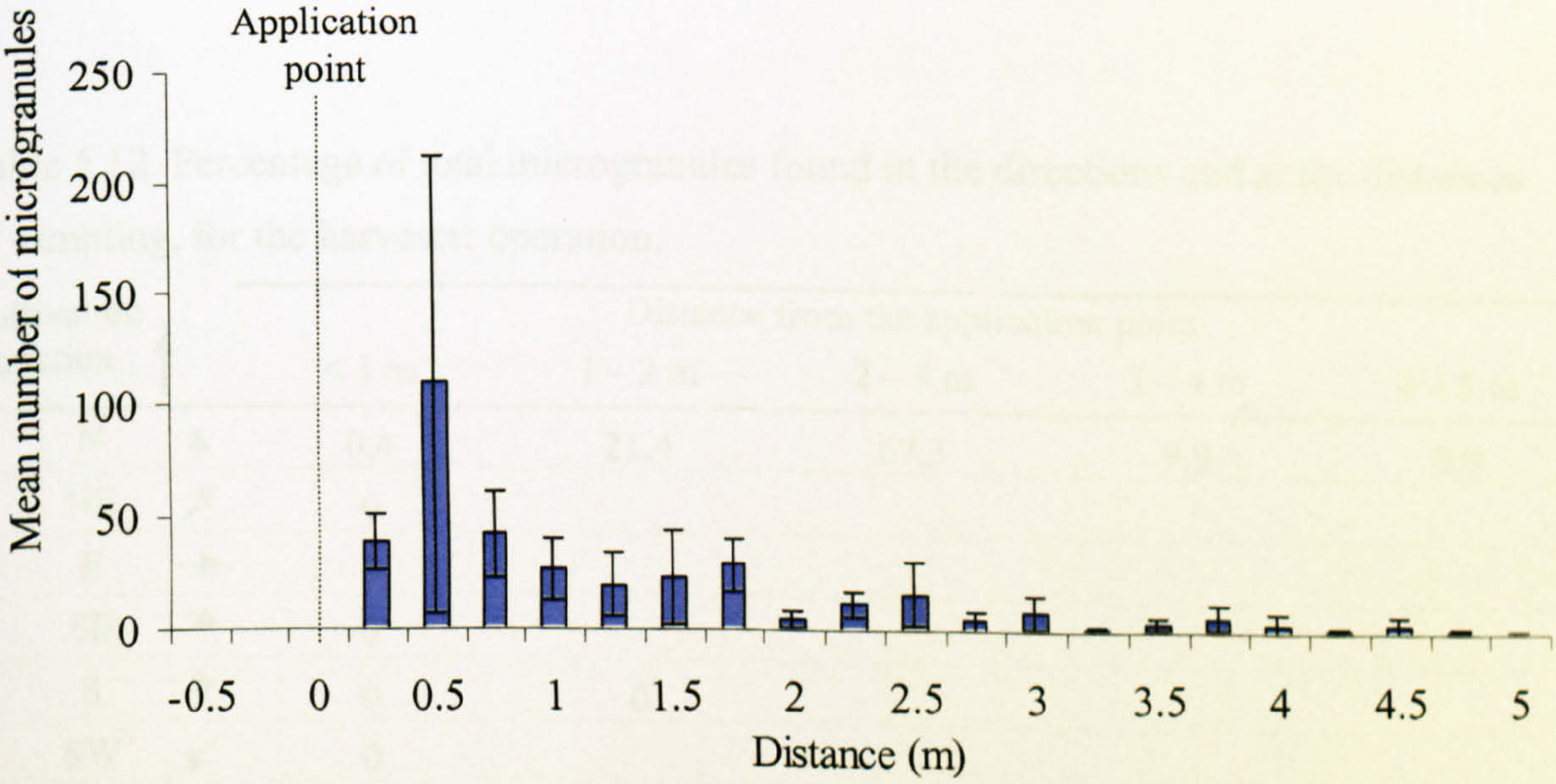


Figure 5.28 Mean number of microgranules in the S to N direction for the planter operation (with +/- SE bars).

Harvester. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.12. All the microgranules

recovered were found past the application point in the direction of cultivation, with 67% of the microgranules moved 2 to 3 m from the application point. The distance with the most microgranules found in was N 2 m, the number of microgranules recovered in the cores relative to this core showed a declining trend. Microgranules were found up to 5 m beyond the application point (Figure 5.29). The action of the harvester is the same as that of the de-stoner; the bed is lifted onto a web where the soil falls back to the ground and the potatoes are removed. The results suggest that the optimum dropping distance of the bed soil was 2 m beyond where it was lifted. This is probably due to the microgranules varying in how far they were lifted along the web before dropping, relative to the speed of the tractor. This distance could be potentially increased on heavy wetter soils.

No lateral movement was found for the harvester. Lateral movement may have taken place but not have been detected using this sampling method. The movement of the microgranules up the bed would result in any lateral movement being further up the bed than could be detected using the compass point sampling pattern.

Table 5.12. Percentage of total microgranules found in the directions and at the distances of sampling, for the harvester operation.

Cultivation Direction ↑	Distance from the application point				
	< 1 m	1 – 2 m	2 – 3 m	3 – 4 m	4 – 5 m
N ↑	0.4	21.4	67.3	9.9	0.9
NE ↗	0				
E →	0				
SE ↘	0				
S ↓	0	0			
SW ↙	0				
W ←	0				
NW ↖	0				

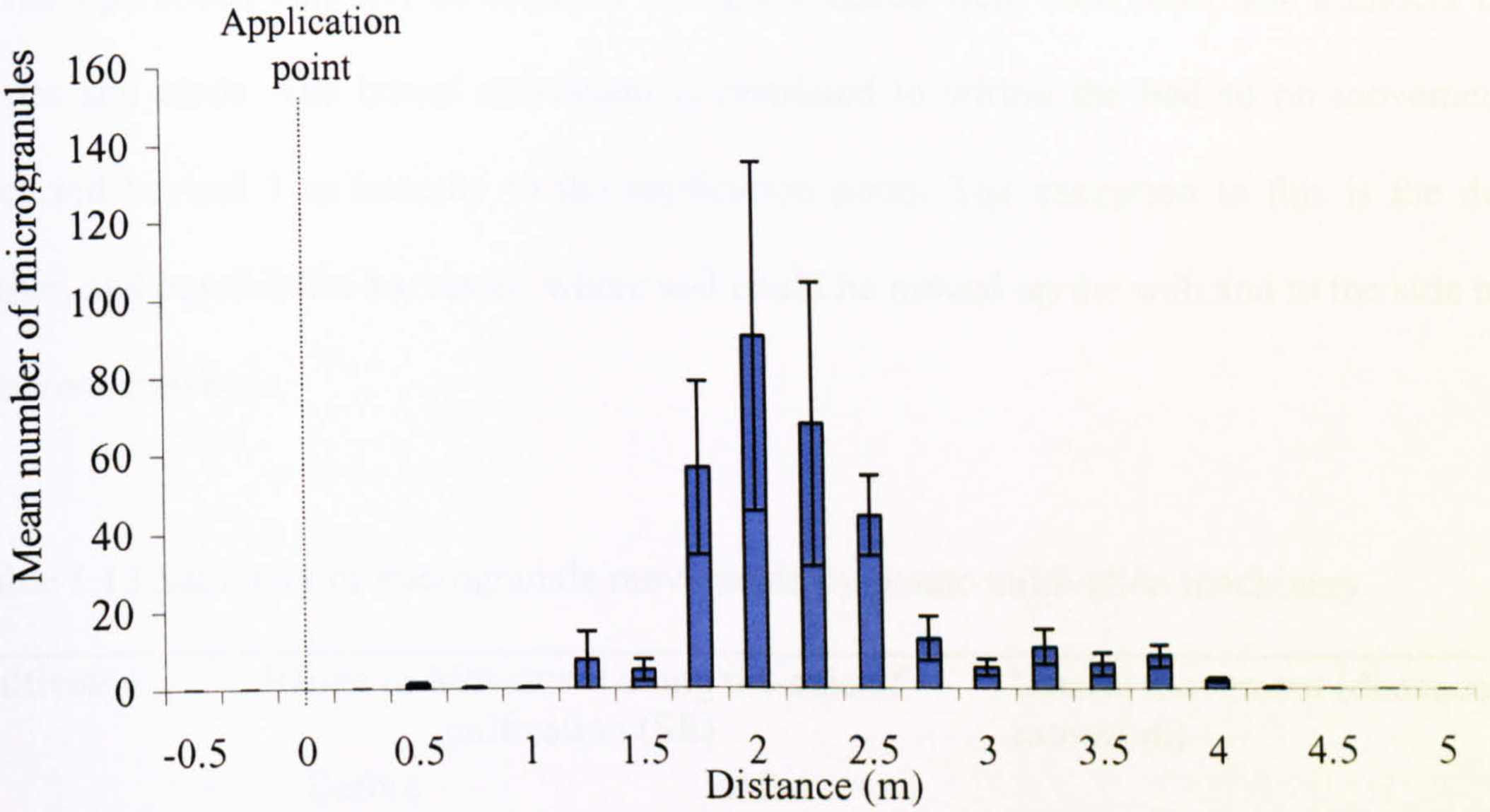


Figure 5.29 Mean number of microgranules in the S to N direction for the harvester operation (with +/- SE bars).

All cultivations involved in potato production caused movement of microgranules, with the exception of the bed-former. The majority of the microgranules were moved along the axis that the cultivation operation passed through the application points, although, most of the cultivations moved the majority of microgranules less than 1 m the spread was up to 5 m. The spread of the microgranules is important due to one cyst being able to start a new infestation point. The range of spread along this axis was significantly different for the separate operations ($p = 0.002$) (Table 5.13). This is to be expected as their functions within a potato crop are different and therefore their cultivation actions are different.

The de-stoner caused the widest spread of microgranules in the bed and the most lateral movement. Although the harvester has a similar action to the de-stoner no movement was found behind the application point, compared with movement of 0.75 m. The reason for this may be the fine tilth of the bed at the harvest point in this experiment, resulting in the soil dropping through the web more rapidly than for the de-stoner.

The harvester and de-stoner were the only operations to cause lateral movement. The de-stoner operations may not be required in light textured field soils with low numbers of stones and clods. The lateral movement is restricted to within the bed so no movement occurred beyond 1 m laterally to the application point. The exception to this is the de-stoner, and possibly the harvester, where soil could be moved up the web and to the side by the cross conveyor.

Table 5.13 Summary of microgranule movements by potato cultivation machinery.

Cultivator	Range of Movement along the axis of cultivation (SE)		Lateral movement (distance moved m)
	Before	After	
Bed tiller	0.33 (0.16)	3.00 (0.14)	None
De-stoner	0.75 (0.0.28)	3.83 (0.22)	NE (0.75) and E (0.25)
Planter	0 (0)	4.75 (0.14)	None
Harvester	0 (0)	4.08 (0.83)	NW (0.75), W (0.25) and SW (0.25)
DF= 3	p =< 0.001		p= 0.450

Potato sequence. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.14. Microgranules were found along the axis of the bed (S to N), and all other directions except E and SE. 46.1% of the microgranules were recovered from NE of the application point. From the samples collected along the bed microgranules were found up to N 9 m and S 1.25 m, with the most found in the 0.25 m N samples (Figure 5.30). The number of microgranules recovered in the cores relative to this core showed an overall declining trend. Lateral movement was found up to the following distances; NE (1 m), NW (1 m), W (0.25 m) and SW (0.5 m) (Table 5.14).

Table 5.14 Percentage of total microgranules found in the directions and at the distances of sampling, for the potato sequence operations.

Cultivation Direction ↑	Distance from the application point									
	< 1	1 –2	2 – 3	3 – 4	4 – 5	5 – 6	6 – 7	7 – 8	8 – 9	9 - 10
N ↑	29.7	1.5	7.9	1.8	5.5	1	1.6	1	0.6	0
NE ↗	1.5									
E →	0									
SE ↘	0									
S ↓	0.5	0.3	0	0	0					
SW ↙	1									
W ←	0.3									
NW ↖	46.1									

Table 5.15 Mean number of microgranules (SE) moved laterally to cultivation direction by potato sequence operations.

Cultivation Direction ↑	Distance from the application point			
	0.25	0.50	0.75	1.0
NE ↗	0	0	0	2.6 (1.3)
NW ↖	48 (35.1)	28.5 (17.5)	3.4 (3.4)	1.3 (1.3)
W ←	0.6 (0.6)	0	0	0
SW ↙	1.1 (0.6)	0.7 (0.3)	0	0

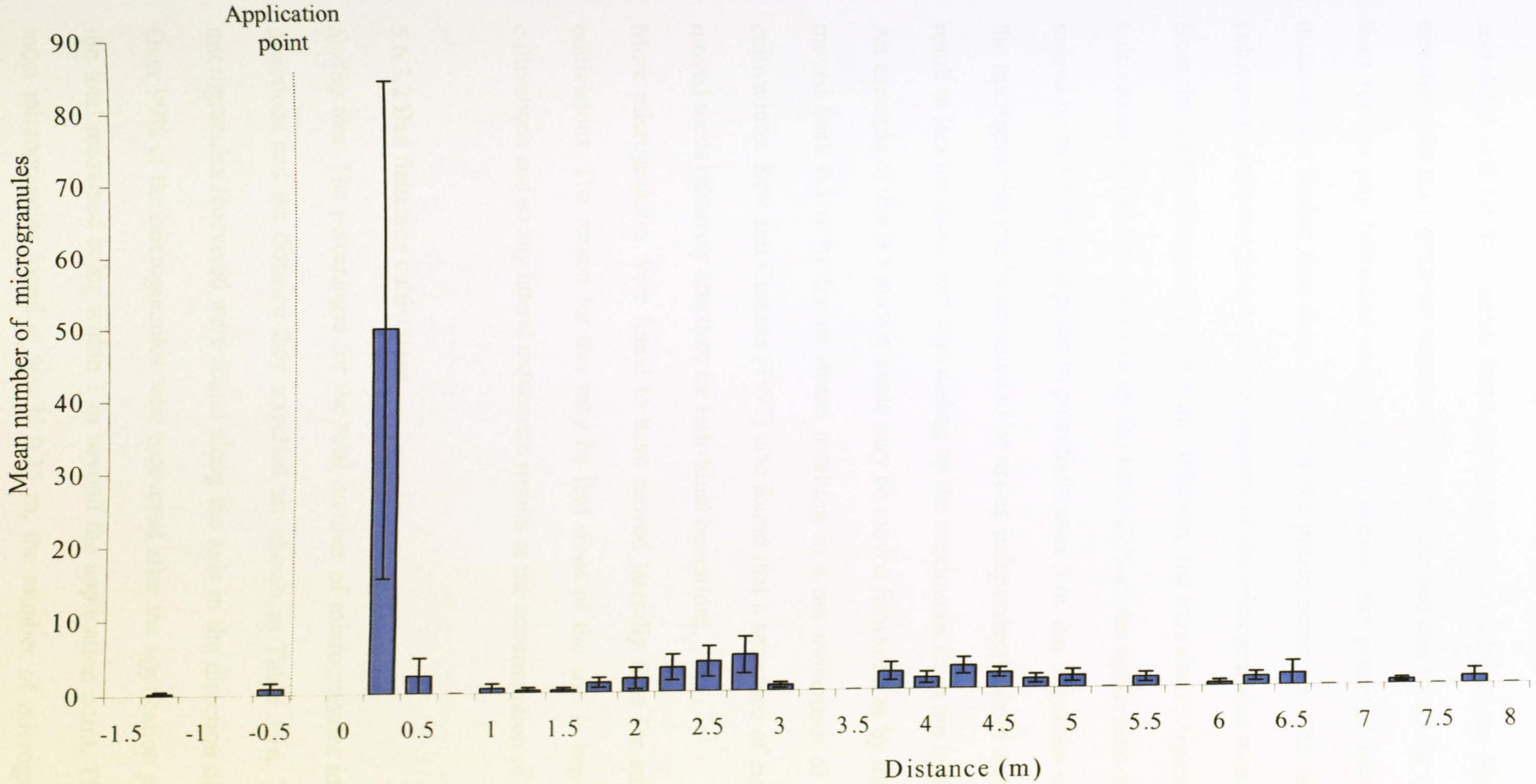


Figure 5.30 Mean number of microgranules in the S to N direction for the potato sequence operations (with +/- SE bars).

The reason for investigating the potato cultivation sequence was to find out if this correlated with the movements found for the individual cultivations. More variation in the directions the microgranules were moved within the bed was found for the potato sequence than for the any individual cultivation. However, the potential cumulative maximum distances are further than those found for the potato sequence. The results of the potato cultivations sequence found that the majority of the microgranules were moved up the bed from the application point, up to 9 m. Whereas, the cumulative figure for the individual cultivations would be over 15 m up the bed. Behind the application microgranules were moved up to 1.5 m, as opposed to potentially over 5 m. An important consideration is that the microgranules can be assumed to be acting independently from each other. This could result in less net movement than adding up the maximums from the individual cultivations. An example of this is a microgranule may be moved forward 2 m by the bed tiller but then moved back 0.5 m by the de-stoner resulting in a net movement of 1.5 m for the two cultivations. Rew and Cussans (1997) also found that a sequence of cultivation operations moved seeds relatively less than for individual operations.

More microgranules were found to have moved laterally than for any of the individual cultivations. The reason for this may be that most of the soil is kept in the bed by the cultivations and so any lateral movement results in the accumulation of microgranules.

5.6.3.2 Bed flattening cultivations

Spring tine. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.16. The majority of the microgranules recovered were found along the axis in the direction of cultivation passed. Over 99% of the microgranules were recovered after the application point, with 95.7% of the total recovered being within 1 m beyond the application point. The distance with the most microgranules found in was N 0.25 m, the number of microgranules recovered at

distances relative to this location showed a declining trend. Microgranules were found in cores up to 2.25 m from the application point in the N direction (Figure 5.31).

The spring tines are fixed (not powered) and this probably explains why no microgranules were moved behind the application. The microgranules from the application would have been dragged with soil by the tines until they were replaced by soil further along the direction of cultivation. This resulted in a decreasing number of microgranules being moved as the distance from the application increased.

Lateral movement was found NW, E and NW up to 0.25 m (Table 5.17). The limited lateral movement is also probably due to the spring tine not being a powered cultivator or having a back roller.

Table 5.16 Percentage of total microgranules found in the directions and at the distances of sampling, for bed flattening using spring tine cultivation.

Cultivation ↑ Direction	Distance from the application point				
	< 1 m	1 – 2 m	2 – 3 m	3 – 4 m	4 – 5 m
N ↑	95.7	2.8	0.6	0	0
NE ↗	0.2				
E →	0.3				
SE ↘	0				
S ↓	0	0			
SW ↙	0				
W ←	0				
NW ↖	0.4				

Table 5.17 Mean number of microgranules (SE) moved laterally to cultivation direction by spring tine operation for bed flattening.

Cultivation ↑ Direction	Distance from the application point			
	0.25	0.50	0.75	1.0
NE ↗	0.3 (0.3)	0	0	0
E →	0.5 (0.5)	0	0	0
NW ↖	0.6 (0.3)	0	0	0

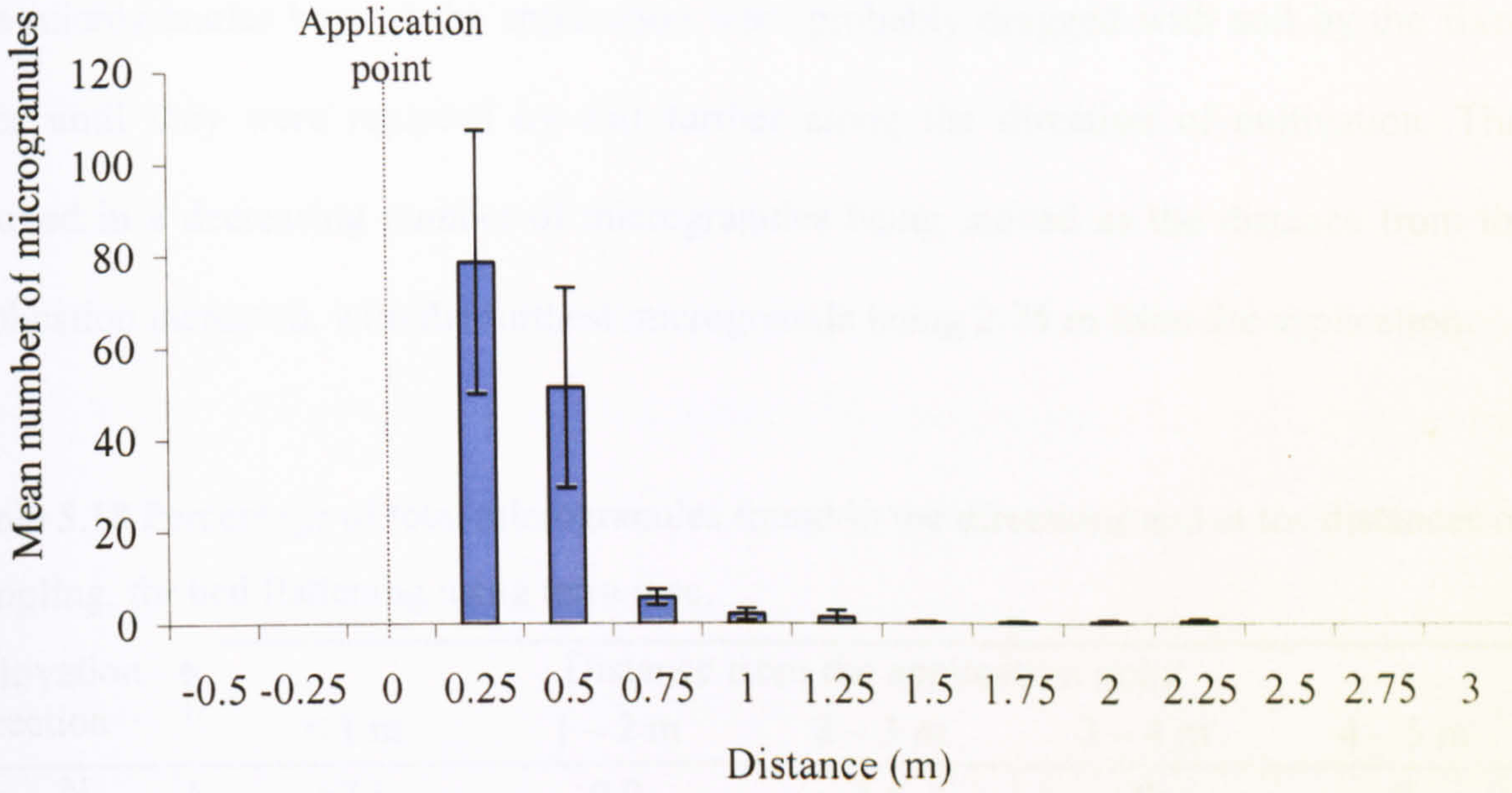


Figure 5.31 Mean number of microgranules in the S to N direction for bed flattening using spring tine cultivation (with +/- SE bars).

Terra-disc. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.18. The majority of the microgranules recovered were found along the axis of cultivation. Over 98% of the microgranules were recovered after the application point, with 67.1% of the total recovered being within 1 m past the application point. The distance with the most microgranules found in was N 0.25 m, the number of microgranules recovered in the cores relative to this core showed a declining trend. Microgranules were found in cores up to 2.75 m from the application point in the N direction and S 0.5 m (Figure 5.32).

The limited movement behind the application point is probably due to the action of the tines on the Smaragd 8, which its manufacturers refer to as 'soil bubbling'. This could result in backward and lateral movement. However, only limited lateral movement was found for this operation. Lateral movement was found in a NW direction up to 0.5 m (Table 5.19). More lateral movement was expected as the bed flattening operation aims to break up the bed and assist in returning soil to the bed furrows. The tines and discs break up the soil, which the rollers flatten out the bed returning soil to the furrows.

The microgranules beyond the application were probably dragged with soil by the fixed tines until they were replaced by soil further along the direction of cultivation. This resulted in a decreasing number of microgranules being moved as the distance from the application increased, with the furthest microgranule being 2.75 m from the application.

Table 5.18 Percentage of total microgranules found in the directions and at the distances of sampling, for bed flattening using terra-disc.

Cultivation Direction ↑	Distance from the application point				
	< 1 m	1 – 2 m	2 – 3 m	3 – 4 m	4 – 5 m
N ↑	67.1	9.9	3.6	0	0
NE ↗	0				
E →	0				
SE ↘	0				
S ↓	9.1	0			
SW ↙	0				
W ←	0				
NW ↖	10.3				

Table 5.19 Mean number of microgranules (SE) moved laterally to cultivation direction by terra-disc operation for bed flattening.

Cultivation Direction ↑	Distance from the application point			
	0.25	0.50	0.75	1.0
NW ↖	7.4 (4.2)	3.9 (3.9)	0	0

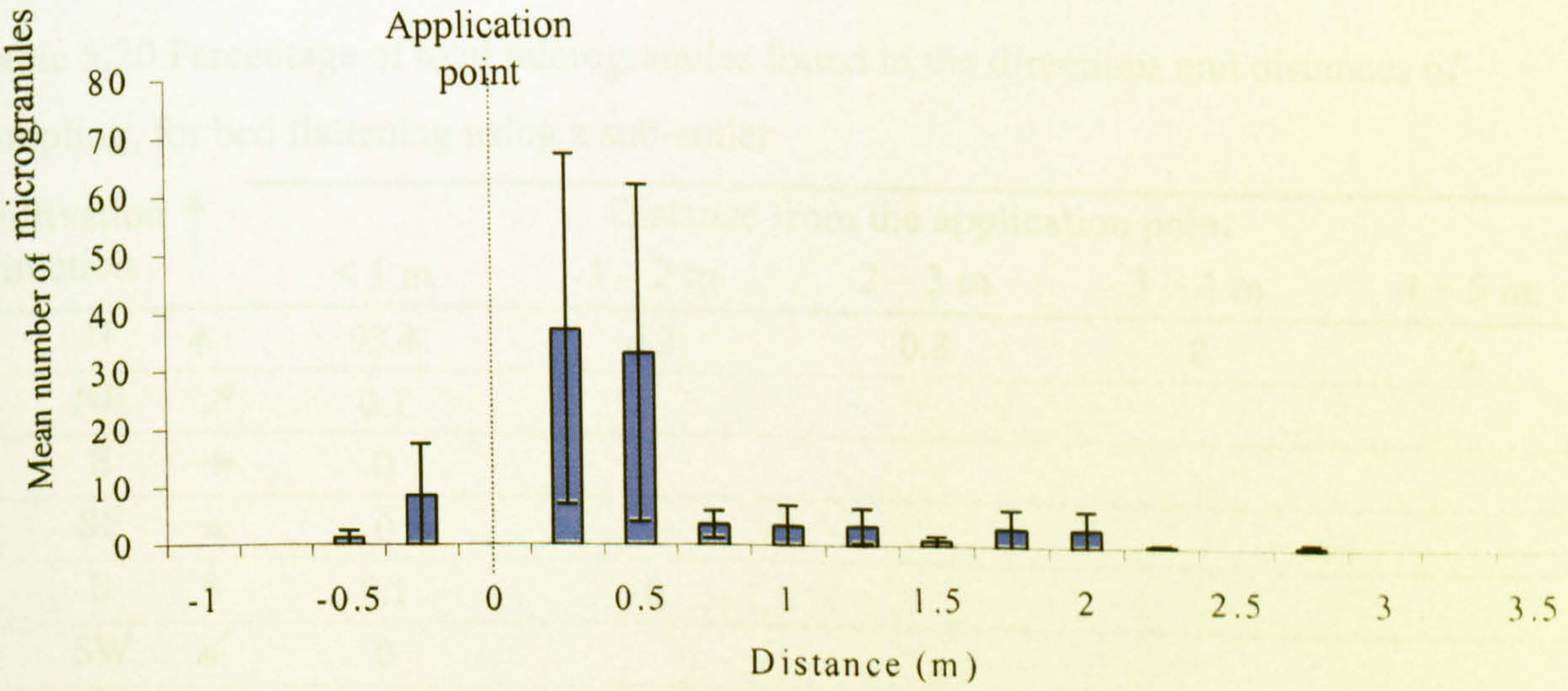


Figure 5.32 Mean number of microgranules in the S to N direction for bed flattening using terra-disc cultivation (with +/- SE bars).

Sub-soiler. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.20. The majority of the microgranules recovered were found along the axis of cultivation. Over 98% of the microgranules were recovered after the application point, with 93.4% of the total recovered being within 1 m past the application point. The distance with the most microgranules found in was N 0.25 m; the number of microgranules recovered in the cores relative to this core showed a declining trend. Microgranules were found in cores up to 2 m from the application point in the N direction (Figure 5.33).

The heavy tines are fixed and this probably explains the limited backward movement from the application. The microgranules past the application point were probably dragged with soil by the tines until they were replaced by soil further along the direction of cultivation. This resulted in a decreasing number of microgranules being moved as the distance from the application increased, with the furthest microgranule being 2 m from the application.

Lateral movement was found up to 0.5 m NW and NE, and 0.25 m W (Table 5.21). The lateral movement was probably microgranules being moved by one tine and picked up by a subsequent one then deposited. The back roller may also have the increased movement from the raised bed down into the furrows.

Table 5.20 Percentage of total microgranules found in the directions and distances of sampling, for bed flattening using a sub-soiler.

Cultivation ↑ Direction	Distance from the application point				
	< 1 m	1 – 2 m	2 – 3 m	3 – 4 m	4 – 5 m
N ↑	93.4	4.3	0.8	0	0
NE ↗	0.1				
E →	0				
SE ↘	0				
S ↓	0.1	0			
SW ↙	0				
W ←	0.1				
NW ↖	1.1				

Table 5.21 Mean number of microgranules (SE) moved laterally to cultivation direction by sub-soiler operation for bed flattening.

Cultivation Direction ↑		Distance from the application point			
		0.25	0.50	0.75	1.0
NW	↖	4.7 (3.1)	0.3 (0.3)	0	0
W	←	0.3 (0.3)			
NE	↗	0.3 (0.3)	0.8 (0.8)		

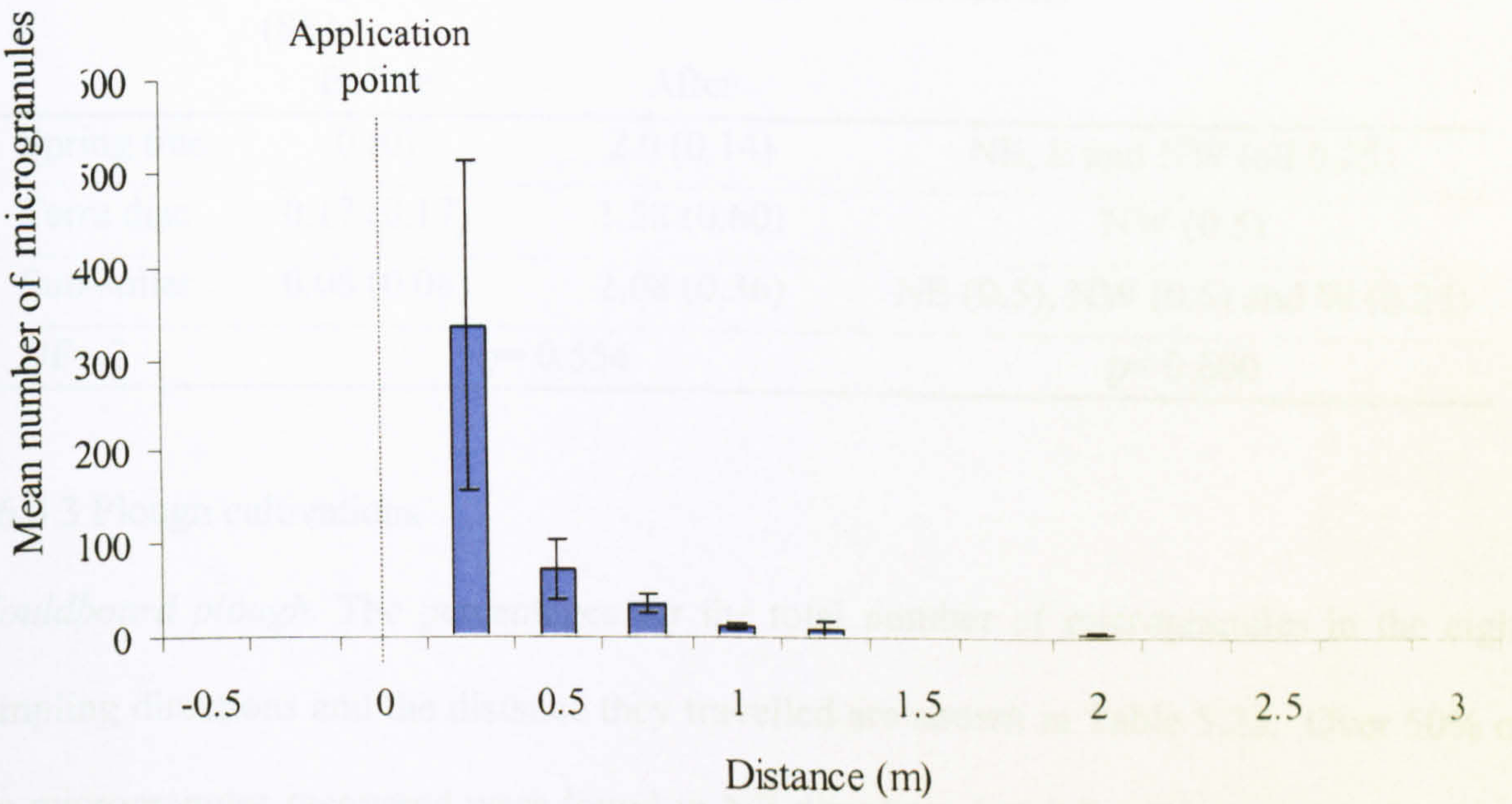


Figure 5.33 Mean number of microgranules in the S to N direction for bed flattening using sub-soiler cultivation (with +/- SE bars).

Table 5.22 shows the directions and distances the microgranules were moved by the three bed flattening cultivations. There was a significant difference between the three cultivation machines used for bed flattening ($p=0.554$ along the axis of cultivation and $p=0.600$ for lateral movement).The cultivation machinery investigated for bed flattening was all non-driven, which resulted in minimal movement of microgranules behind the application point. The Terra-disc cultivator moved the microgranules least along the axis of cultivation (1.58 m). However, it also produced the least lateral movement, which is the purpose of the bed flattening operation. This suggests that although it would result in cysts being moved less, if utilised for bed flattening, it is not as suited as the other cultivators for this

operation. The other cultivations caused microgranule movement over 2 m, past the application point, but also caused lateral movement.

Table 5.22 Summary of distances and directions microgranules were moved by the bed flattening cultivations

Cultivator	Mean range of movement (m) along the direction of cultivation (SE)		Lateral movement (distance moved m)
	Before	After	
Spring tine	0 (0)	2.0 (0.14)	NE, E and NW (all 0.25)
Terra disc	0.17 (0.17)	1.58 (0.60)	NW (0.5)
Sub-soiler	0.08 (0.08)	2.08 (0.36)	NE (0.5), NW (0.5) and W (0.25)
DF= 2	p= 0.554		p= 0.600

5.6.3.3 Plough cultivations

Mouldboard plough. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.23. Over 50% of the microgranules recovered were found in NE direction. Lateral movement was found in the NW and W direction at 0.25 m, and up to 0.5 m in the E and NE directions (Table 5.24).

The majority of the movement was to the side where the soil was being inverted, the East. This was expected as some lateral movement can be seen when using a mouldboard plough. Movement was also found opposite to that where the soil was being inverted; this movement was not expected as the plough is designed to cut through the soil and invert to one side. It may be due to the microgranules being pushed by the side of plough away from inversion. These results conflict with the findings of Marshall and Brain (1999) who found that seeds were only moved laterally in the direction of inversion. However, their application of microgranules was only on the surface which may have resulted in the different findings. The distance with the most microgranules found in the S to N axis was at 0.25 m N; the number of microgranules recovered in the cores after this location showed

a declining trend. Microgranules were found in cores up to 1 m from the application point in the N direction (Figure 5.34). The limited forward movement of microgranules is due to the mouldboards inverting the soil laterally and therefore causing limited dragging in the direction of cultivation. The mouldboard plough has no mechanised parts during cultivation, this is probably the reason for no microgranules being moved behind the application.

Table 5.23 Percentage of total microgranules found in the directions and at the distances of sampling for mouldboard plough.

Cultivation Direction ↑	Distance from the application point				
	< 1 m	1 – 2 m	2 – 3 m	3 – 4 m	4 – 5 m
N ↑	24.1	0.9	0	0	0
NE ↗	50.3				
E →	21.4				
SE ↘	0				
S ↓	0	0			
SW ↙	0				
W ←	0.3				
NW ↖	3.4				

Table 5.24 Mean number of microgranules (SE) moved laterally to cultivation direction by mouldboard plough operation.

Cultivation direction ↑	Distance from the application point			
	0.25	0.50	0.75	1.0
NE ↗	48.8 (35.8)	9.9 (6.2)	0	0
E →	15.3 (11.7)	9.5 (5.7)	0	0
NW ↖	3.4 (3.4)	0	0	0
W ←	0.4 (0.4)	0	0	0

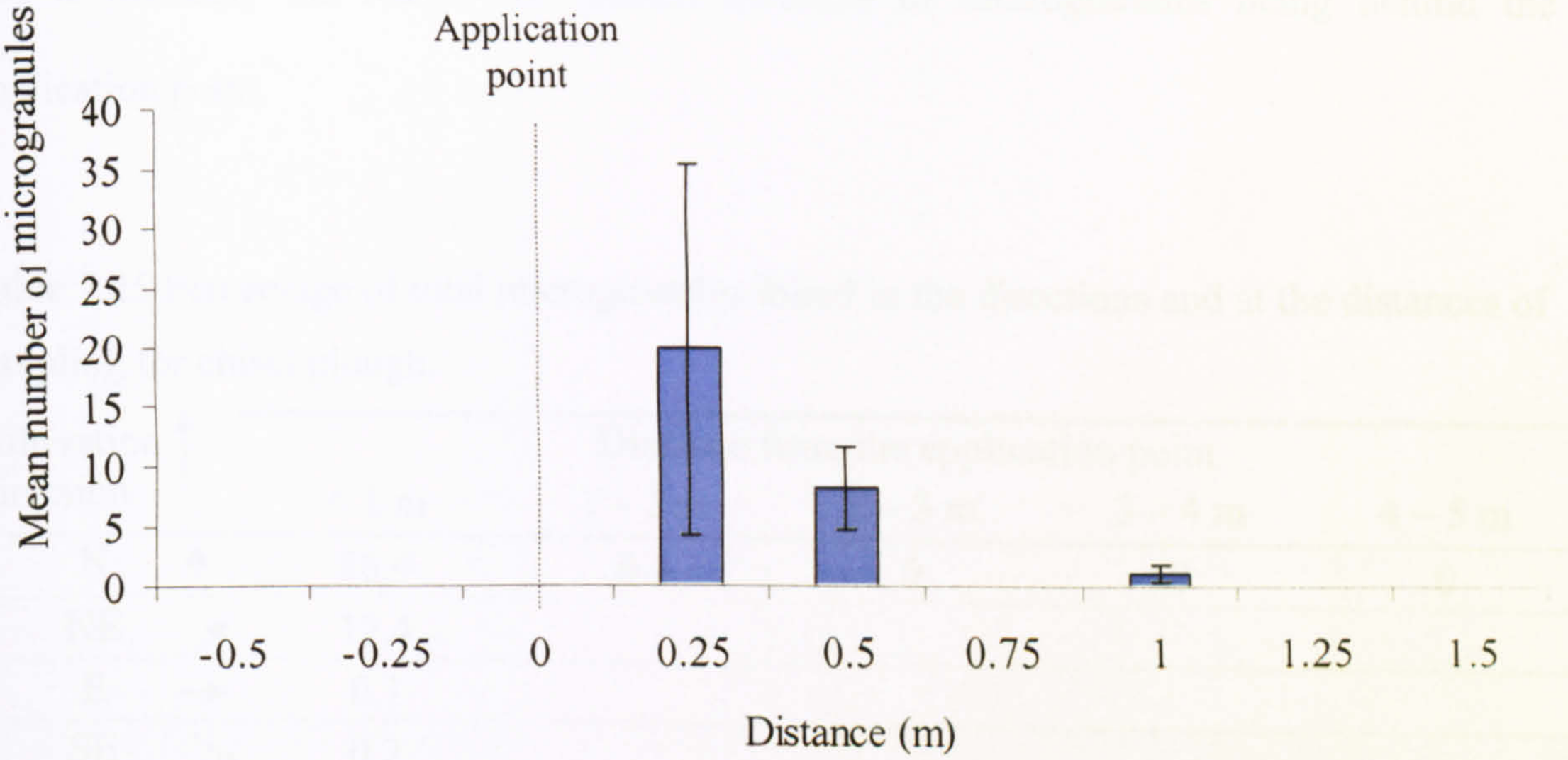


Figure 5.34 Mean number of microgranules in the S to N direction using mouldboard plough (with +/- SE bars).

Chisel plough. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.25. The majority of the microgranules recovered were found along the axis of cultivation. 86.8% of the microgranules were recovered after the application point, with 86.4% of the total recovered being within 1 m N of the application point; no microgranules were found in the S direction. The distance with the most microgranules found was N 0.25 m; the number of microgranules recovered in the cores relative to this core showed a declining trend. Microgranules were moved up to 1.25 m past the application point (Figure 5.35). Microgranules were moved in all lateral directions at 0.25 m and 0.50 for NE and SW (Table 5.26). This movement was probably microgranules being moved by one tine and picked up by a subsequent one then deposited.

The chisel plough movements were similar to the sub-soiler and terra-disc, they all have heavy tines to work the soil. The limited forward movement of microgranules (up to 1.75 m) in relation to the terra-disc and sub-soiler may have been due to the other cultivators

having back rollers attached. The chisel plough has no mechanised parts during cultivation; this is probably the reason for limited numbers of microgranules being behind the application point.

Table 5.25 Percentage of total microgranules found in the directions and at the distances of sampling for chisel plough.

Cultivation Direction ↑	Distance from the application point				
	< 1 m	1 – 2 m	2 – 3 m	3 – 4 m	4 – 5 m
N ↑	86.4	0.4	0	0	0
NE ↗	12.4				
E →	0.1				
SE ↘	0.2				
S ↓	0	0			
SW ↙	1.1				
W ←	0				
NW ↖	0.5				

Table 5.26 Mean number of microgranules (SE) moved laterally to cultivation direction by chisel plough operation.

Cultivation Direction ↑	Distance from the application point			
	0.25	0.50	0.75	1.0
NE ↗	91.2 (72.4)	1.9 (1.9)	0.4 (0.4)	0
E →	0.3 (0.3)	0	0	0
SE ↘	1.2 (1.2)	0	0	0
NW ↖	3.6 (1.9)	0	0	0
SW ↙	0.8 (0.8)	0.3 (0.3)		0

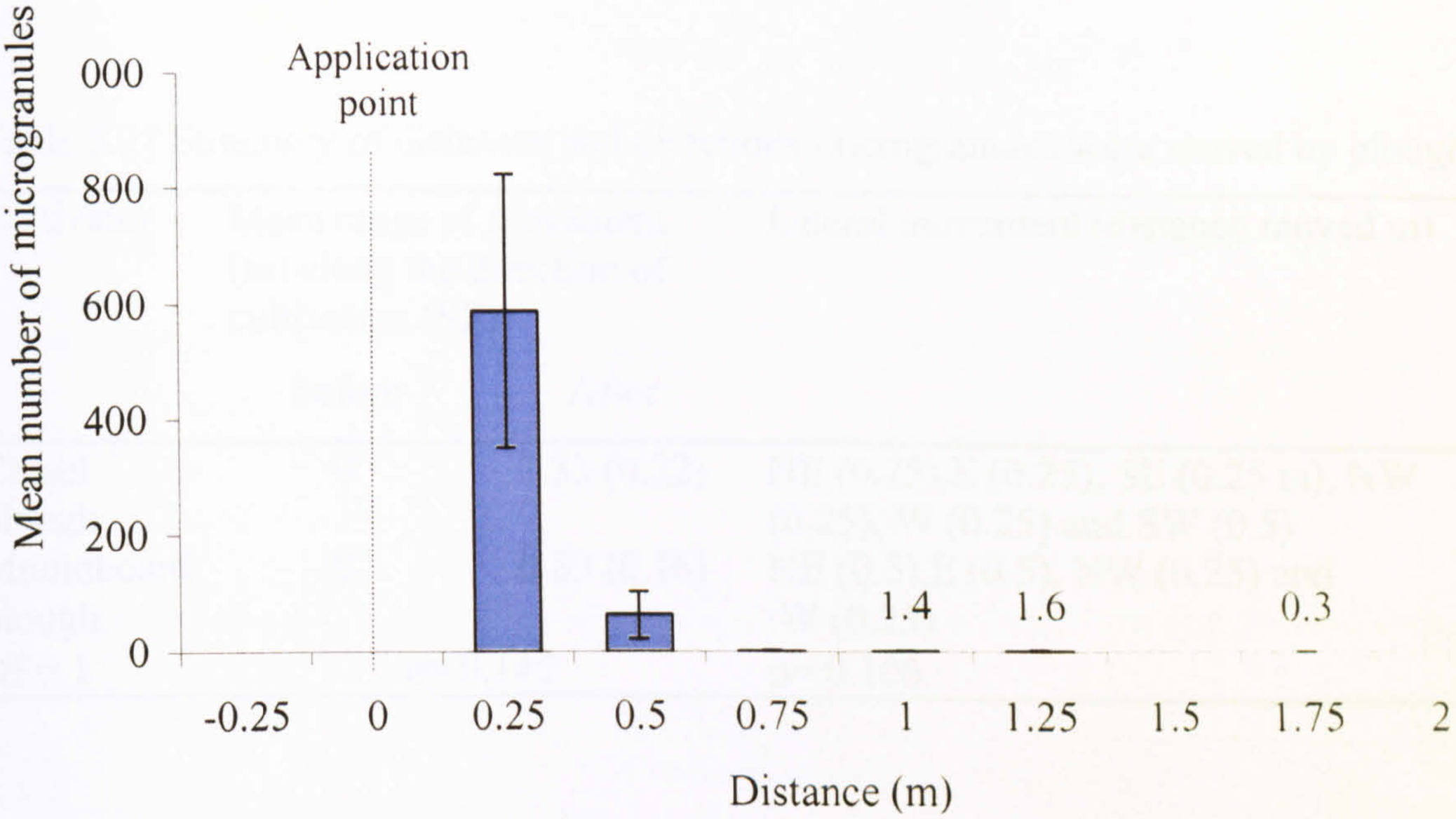


Figure 5.35 Mean number of microgranules in the S to N direction using chisel plough (with +/- SE bars).

The two types of plough were found to be significantly different in terms of the distance and direction that they moved the microgranules (Table 5.27). The chisel plough moved the microgranules in more directions and to greater distances than the mouldboard plough. This is to be expected due the differences in the mode of action of the cultivator. The chisel plough breaks up the soil as it is dragged through it, resulting in soil and microgranule being caught up and moved on the tines. The mouldboard plough in contrast is an angled cultivator that inverts the soil to one side as it moves through the soil. This results in the minimal drag found in this work. The limited distances of movement of the microgranules by mouldboard ploughing was also found by Marshall and Brain (1999); they found that the use of a mouldboard plough in a cultivation sequence limited horizontal seed movement.

Table 5.27 Summary of distances and directions microgranules were moved by ploughing.

Cultivator	Mean range of movement (m) along the direction of cultivation (SE)		Lateral movement (distance moved m)
	Before	After	
Chisel plough	0	1.33 (0.22)	NE (0.75),E (0.25), SE (0.25 m), NW (0.25), W (0.25) and SW (0.5)
Mouldboard plough	0	0.83 (0.16)	NE (0.5),E (0.5), NW (0.25) and W (0.25)
DF= 1	p= 0.145		p= 0.106

5.6.3.4 Cereal Cultivations

Spring tine. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.28. The majority of the microgranules recovered were found along the axis in the direction of cultivation. Over 97% of the microgranules were recovered after the application point, with 86.4% of the total recovered being within 1 m of the application point. The distance with the most microgranules found was N 0.25 m; the number of microgranules recovered in the cores relative to this core showed a declining trend. Microgranules were found in cores up to 1.25 m from the application point in the N direction, and up to S 0.25 m (Figure 5.36).

The spring tines are fixed but do spring back as they are pulled through the soil; the result of this was that a limited number of microgranules were moved backwards. This was not found when using the spring tine for the bed flattening operation. The reason for this may be differences in the resistance of the soil. The bed flattening operation followed several intensive cultivations on the bed, which formed a fine tilth, whereas the spring tine followed mouldboard ploughing for the cereal experiment. The microgranules from the application were probably dragged with soil by the tines until they were replaced by soil further along the direction of cultivation, or were released when the tine sprang back. This resulted in a decreasing number of microgranules past the application increased, with the

furthest microgranule being 2.5 m from the application, compared with 2.25 for the bed flattening spring tine operation.

Microgranules were moved laterally NE and NW at 0.25 m (Table 5.29). The lateral movement was probably microgranules being moved by one tine and picked up by a subsequent one then deposited.

Table 5.28 Percentage of total microgranules found in the directions and at the distances of sampling for spring tine.

Cultivation ↑ Direction	Distance from the application point				
	< 1 m	1 – 2 m	2 – 3 m	3 – 4 m	4 – 5 m
N ↑	78.1	3.5	0.5	0	0
NE ↗	0.1				
E →	0				
SE ↘	0				
S ↓	17.7	0			
SW ↙	0				
W ←	0.1				
NW ↖	0				

Table 5.29 Mean number of microgranules (SE) moved laterally to cultivation direction by spring tine operation.

Cultivation ↑ Direction	Distance from the application point			
	0.25	0.50	0.75	1.0
NE ↗	0.3 (0.3)	0	0	0
NW ↖	0.6 (0.6)	0	0	0

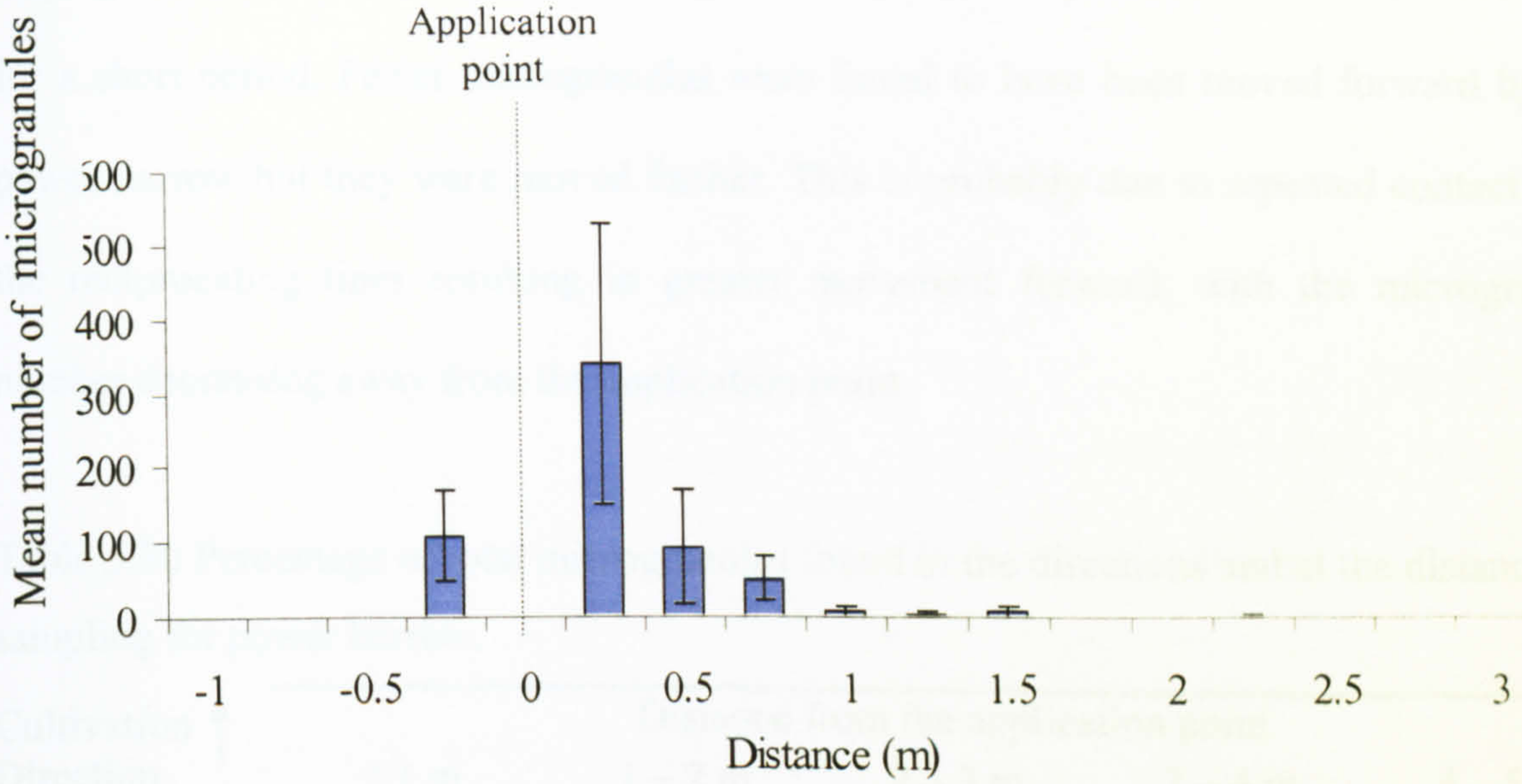


Figure 5.36 Mean number of microgranules in the S to N direction using spring tine (with +/- SE bars).

Power harrow. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.30. Along the S to N axis over 50% of the microgranules were recovered. The cores found to have the most microgranules were at 0.25 m; the number of microgranules recovered in the cores relative to this location showed a declining trend. Microgranules were found in cores up to 3 m from the application point in the N direction and up to 0.75 m S (Figure 5.37). Microgranules were found in all lateral directions at 0.25 m except for SE. W and SW directions had microgranules moved to 0.5 m, NW to 0.75 m and NE to 0.75 m (Table 5.31). The most microgranules were found at 0.25 m SW.

The spatial pattern of the microgranules showed that the microgranules were moved in most directions by power harrow. The harrow is made up of reciprocating twin tines that are powered by the tractor engine. This results in the microgranules from the application point being agitated out in all directions, with most being moved behind the application point Although most of the microgranules were moved behind, they were only moved a relatively short distance. This is similar to the bed tiller where the number of

microgranules moved backwards was high but they were only in contact with the cultivator for a short period. Fewer microgranules were found to have been moved forward by the power harrow but they were moved further. This is probably due to repeated contact with the reciprocating tines resulting in greater movement forward, with the microgranule number decreasing away from the application point.

Table 5.30 Percentage of total microgranules found in the directions and at the distances of sampling for power harrow.

Cultivation ↑ Direction	Distance from the application point				
	< 1 m	1 – 2 m	2 – 3 m	3 – 4 m	4 – 5 m
N ↑	8.9	4.6	1	0.2	0
NE ↗	3.6				
E →	0.4				
SE ↘	0				
S ↓	34	0			
SW ↙	38.6				
W ←	5.6				
NW ↖	3.2				

Table 5.31 Mean number of microgranules (SE) moved laterally to cultivation direction by power harrow operation

Cultivation ↑ Direction	Distance from the application point			
	0.25	0.50	0.75	1.0
NE ↗	6.5 (3.3)	4.3 (2.8)	0.8 (0.4)	0
E →	1.3 (1.3)	0	0	0
NW ↖	5 (2.5)	0.8 (0.8)	0.4 (0.4)	4 (4.0)
W ←	17.2 (10.8)	0.8 (0.4)	0	0
SW ↙	123 (74.3)	1.3 (0.8)	0	0

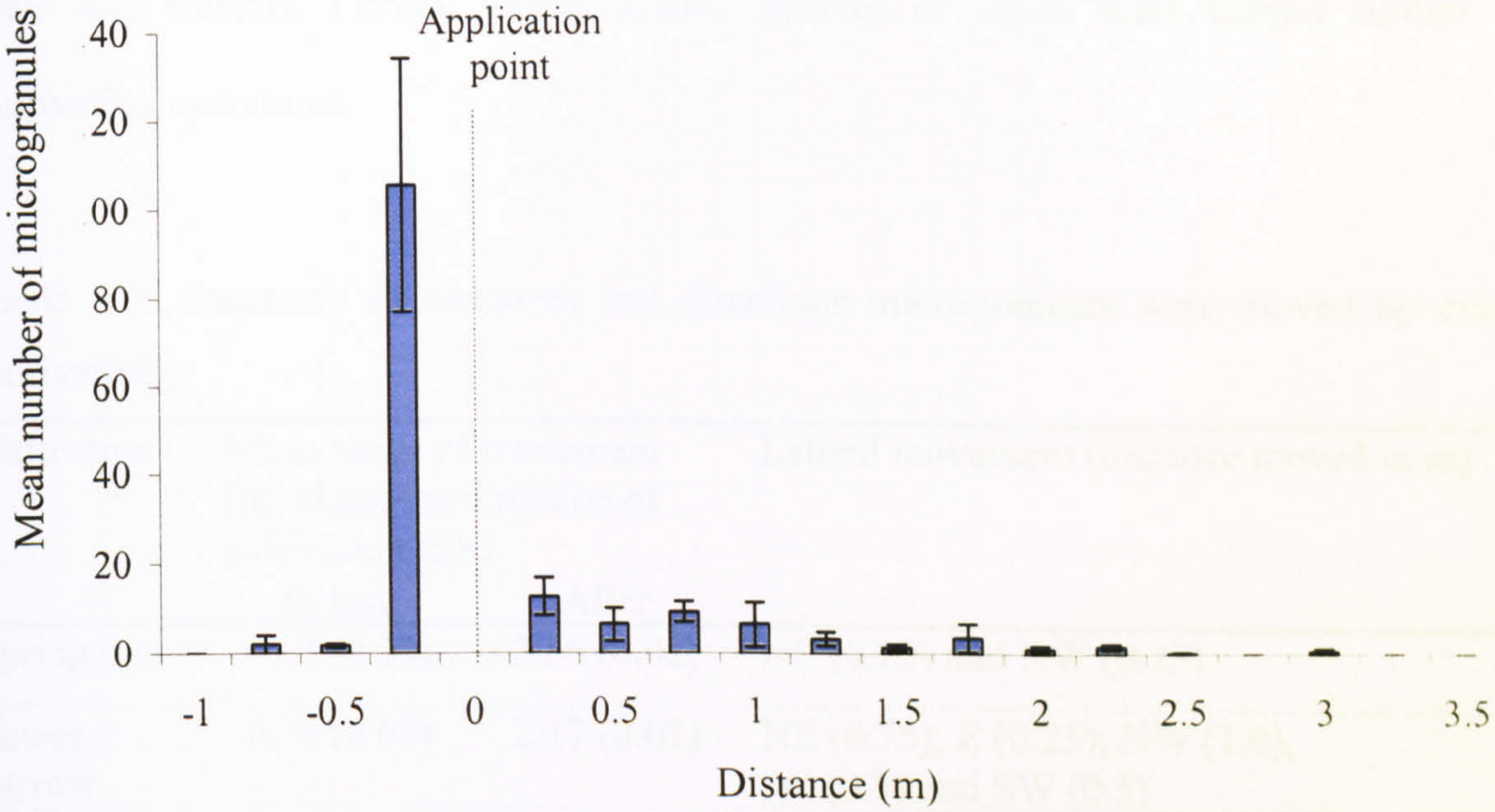


Figure 5.37 Mean number of microgranules in the S to N direction using power harrow (with +/- SE bars).

There was a significant difference between the movement of the microgranules by the two cereal cultivators (Table 5.32). The spring tine moved the microgranules further than the power harrow along the axis of cultivation. Rew and Cussans (1997) also found that the spring tines moved seeds further than the power harrow. However, Grundy, Mead and Burston (1999) in a similar study found that the power harrow moved seeds the furthest. Rew and Cussans (1997) used oilseed rape seeds which are closer in size to the microgranules used in this experiment than the plastic beads used by Grundy, Mead and Burston (1999).

The power harrow caused more lateral movement than the spring tine, which was expected due to the power harrow being a powered cultivation, although, Marshall and Brain (1999) found lateral movement in all directions by the spring tine. The movements found for the cereal cultivations were greater than those found for the studies investigating seed movement (Rew and Cussans,1997; Grundy, Mead and Burston, 1999; Marshall and Brain, 1999). A possible reason for the greater movement is that PCN cysts are smaller than the seed species investigated in the seed movement studies. This concurs with the findings of

Rew and Cussans (1997), where smaller species of seeds were moved further by cultivation operations.

Table 5.32 Summary of distances and directions microgranules were moved by cereal cultivations.

Cultivator	Mean range of movement (m) along the direction of cultivation (SE)		Lateral movement (distance moved in m)
	Before	After	
Spring tine	0.58 (0.08)	2.58 (0.02)	NE (0.25) and NW (0.25)
Power harrow	0.08 (0.08)	2.17 (0.02)	NE (0.75), E (0.25), NW (1.0), W (0.5) and SW (0.5)
DF= 1	p= 0.789		p= 0.071

Winter wheat sequence. The results are for the microgranules recovered after winter wheat cultivation sequence; this consisted of Terra-disc, spring tine then power harrow. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.33. The majority of the microgranules recovered were found along the axis of the cereal cultivations (S to N). Over 55% of the microgranules were recovered after the application point, with 40.9% of the total recovered being within 1 m past the application point. The distance with the most microgranules found in was N 0.5 m; the number of microgranules recovered in the cores relative to this core showed a declining trend along this axis. Microgranules were found in cores up to N 4.5 m and S 0.25 m (Figure 5.38). Lateral movement was found up to 2 m for directions NE and E, with SE having microgranules found up to 1.25 m. Movement also occurred in the three W directions; SW and W to 0.5 m and NW to 0.75 m (Table 5.34).

Table 5.33 Percentage of total microgranules found in the directions and at the distances of sampling for winter wheat cultivation sequence.

Cereal cultivations direction ↑	Distance from the application point									
	< 1	1 – 2	2 – 3	3 – 4	4 – 5	5 – 6	6 – 7	7 – 8	8 – 9	9 -10
N ↑	40.9	13.4	4.2	1.1	0.6	0	0	0	0	0
NE ↗	2	0								
E →	4	0								
SE ↘	0.8	0								
S ↓	0.1	0	0	0						
SW ↙	8.1	1.5	0	0	0					
W ←	7.4	10.2	0	0	0					
NW ↖	2.6	0.2	0	0	0					

Table 5.34 Mean number of microgranules (SE) moved laterally to the cereal cultivation direction for the winter wheat sequence.

Cereal cultivations Direction ↑	Distance from the application point							
	0.25	0.5	0.75	1.0	1.25	1.50	1.75	2.00
NE ↗	8.9 (5.1)	0.3 (0.3)	0	0	0	0	0	0
E →	18.2 (12.3)	0.7 (0.3)	0	0	0	0	0	0
SE ↘	0.4 (0.4)	2.8 (2.1)	0.5 (0.5)	0	0	0	0	0
SW ↙	18 (5.8)	16.4 (8.9)	3.6 (2.0)	2.1 (1.1)	4.7 (3.1)	0.6 (0.3)	1 (1)	0.4 (0.4)
W ←	6.2 (5.5)	15.5 (15.5)	13 (13)	11 (11)	1 (1)	42.5 (33)	3.7 (3.7)	0.5 (0.5)
NW ↖	6.5 (6.5)	2.4 (2.4)	3.2 (3.2)	0.7 (0.7)	1 (1)	0	0	0

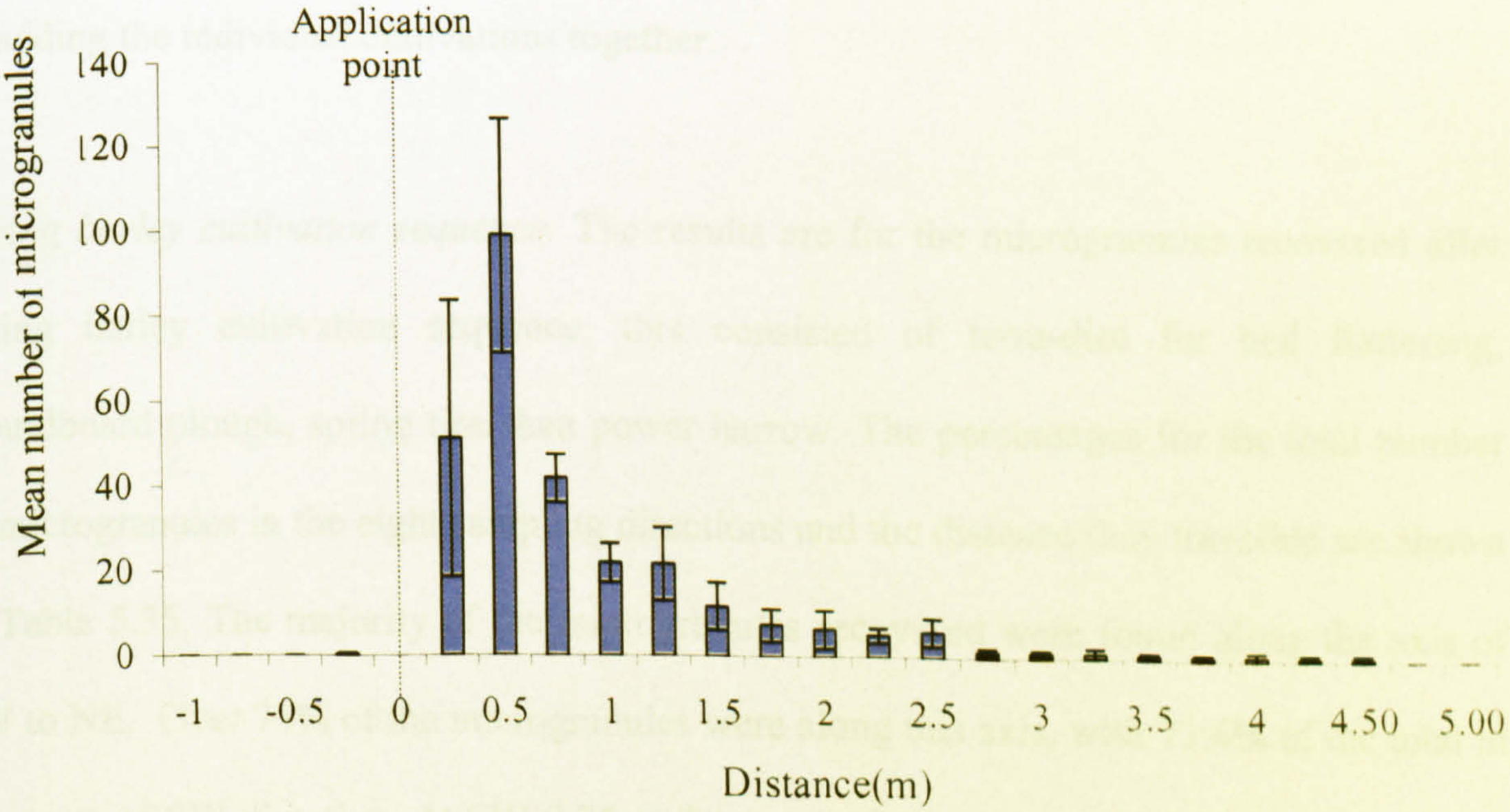


Figure 5.38 Mean number of microgranules in the S to N direction for the winter wheat cultivation sequence (with +/- SE bars).

Extensive movement was found from the application point with this sequence of cultivations. The nature of the sequence was to mimic the seedbed preparation for winter wheat after potatoes. The beds first have to be flattened, but due to the working of the soil by the potato harvesting operation no ploughing may be necessary before using the spring tines and power harrow. The field is cultivated for cereals after potatoes at right angles to that of the potato crop. By placing the application point in the plot prior to the bed flattening the sequence resulted in sampling for movement of the microgranules moved relative to two cultivation directions. The result was that microgranules were moved in all directions from the application point.

The terra-disc, as an individual cultivation, moves microgranules over 2 m past the application point. The microgranules having been moved by this operation would then acted on from their new location by the subsequent operations at right angles, resulting in a large spread of microgranules

Most microgranule movement was past the application in the direction of the spring tine and power harrow cultivations these showed a declining trend up to 4.5 m. The distances moved as with the potato sequence were not as far as the potential cumulative movement of adding the individual cultivations together.

Spring barley cultivation sequence. The results are for the microgranules recovered after spring barley cultivation sequence, this consisted of terra-disc for bed flattening, mouldboard plough, spring tine then power harrow. The percentages for the total number of microgranules in the eight sampling directions and the distance they travelled are shown in Table 5.35. The majority of the microgranules recovered were found along the axis of SW to NE. Over 75% of the microgranules were along this axis, with 73.4% of the total in the cores of SW direction. At SW 0.75 m the most microgranules were found, the number of microgranules recovered in the cores relative to this core showed a declining trend

(Figure 5.39). In the direction of all cultivations excluding the bed flattening operation (i.e. S to N) the distance with the most microgranules found was 0.5 m N, the number of microgranules recovered in the cores relative to this core showed a declining trend. Microgranules were found in cores up to 3.75 m from the application point in the N direction and S 0.75 m (Figure 5.40). Microgranules were found in all the lateral directions with the exception of E (Table 5.36).

Table 5.35 Percentage of total microgranules found in the directions and at the distances of sampling for spring barley cultivation sequence.

Cereal cultivations direction ↑	Distance from the application point									
	< 1	1 – 2	2 – 3	3 – 4	4 – 5	5 – 6	6 – 7	7 – 8	8 – 9	9 -10
N ↑	3.3	0.2	0.9	0.8	0	0	0	0	0	0
NE ↗	3.6	0								
E →	0	0								
SE ↘	3.6	0								
S ↓	1.4	0								
SW ↙	67.1	6.3								
W ←	8.5	0.1								
NW ↖	3.7	0								

Table 5.36 Mean number of microgranules (SE) moved laterally to cultivation direction by spring barley cultivation sequence.

Cereal Cultivations Direction ↑	Distance from the application point							
	0.25	0.5	0.75	1.0	1.25	1.50	1.75	2.00
NE ↗	6.0 (3.2)	2.8 (1.6)	0.5 (0.5)	0	0	0	0	0
SE ↘	2.2 (0.4)	7.0 (7)	0	0	0	0	0	0
SW ↙	36.3 (20)	66.9 (32.8)	69.1 (36.8)	8.4 (3.4)	5.2 (3.3)	2.2 (1.3)	0.4 (0.4)	0
W ←	8.6 (2.5)	10.9 (1.9)	2.4 (0.9)	1.3 (0.9)	0	0.3 (0.3)	0	0
NW ↖	5.7 (2.9)	3.0 (1.8)	0.9 (0.9)	0	0	0	0	0

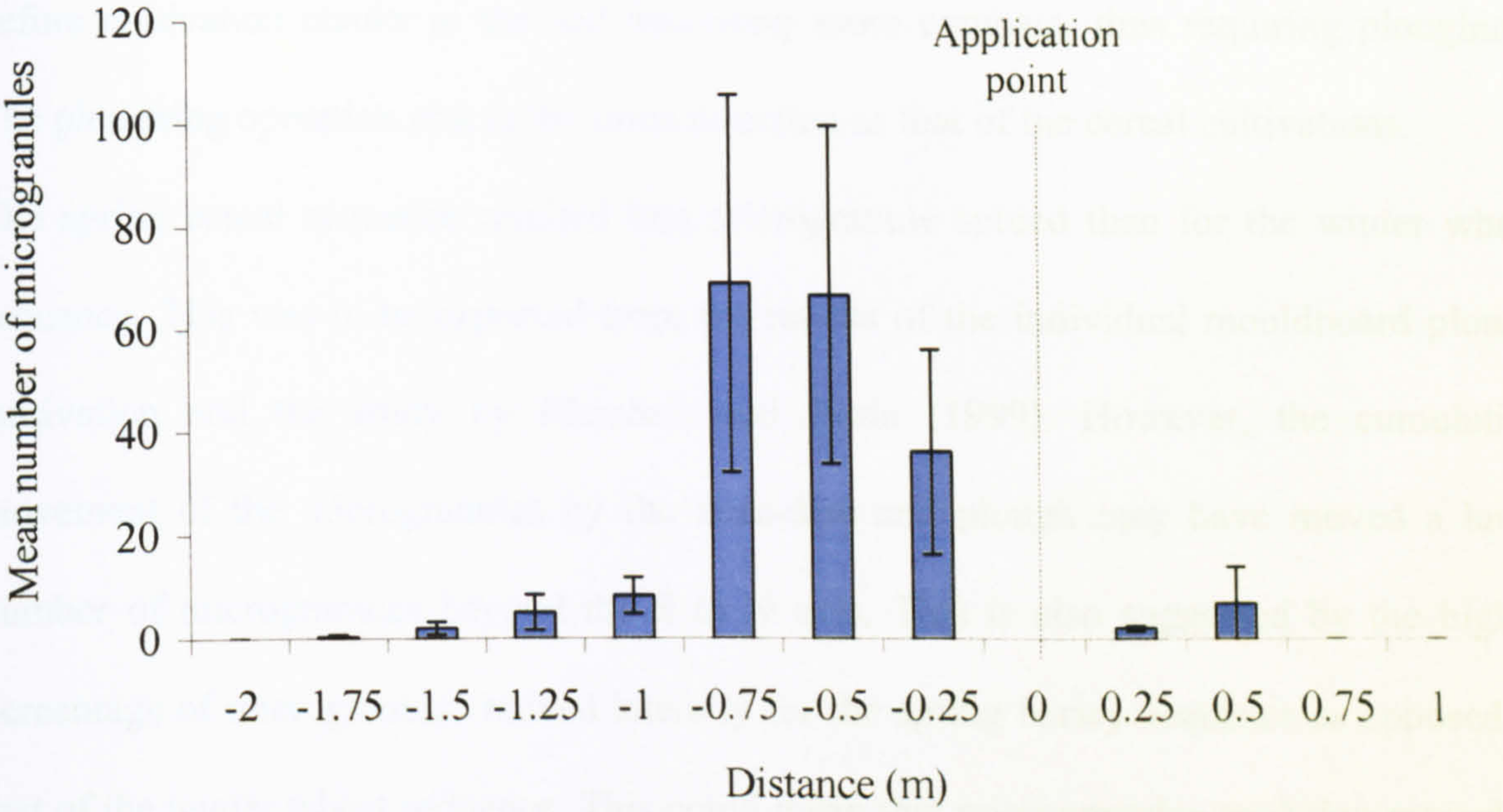


Figure 5.39 Mean number of microgranules in the SW to NE direction for spring barley cultivation sequence (with +/- SE bars).

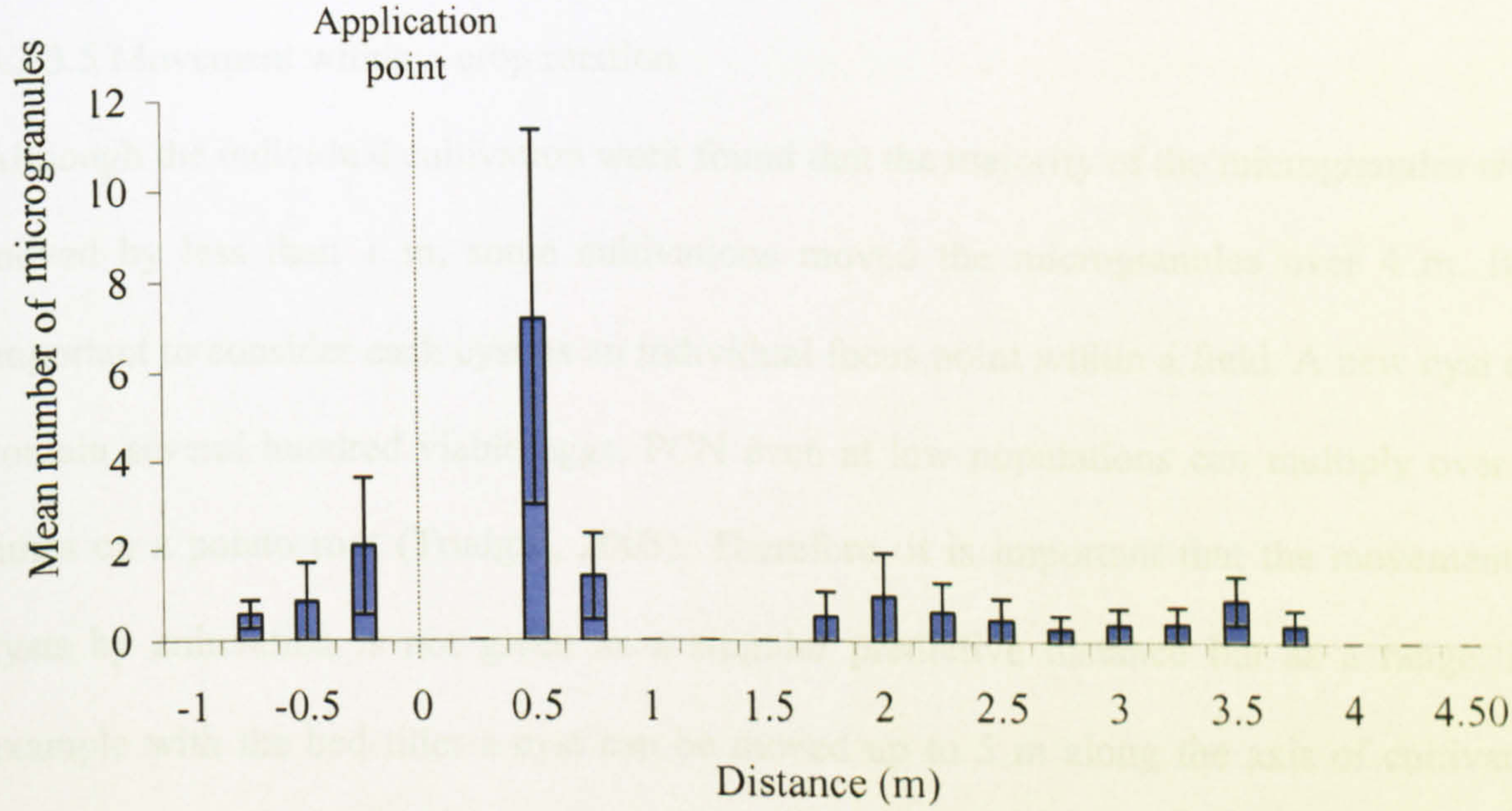


Figure 5.40 Mean number of microgranules in the S to N direction for spring barley cultivation sequence (with +/- SE bars).

This sequence was the same as the winter wheat, except with the addition of the mouldboard plough. The reason for this was to simulate the seedbed preparation after potatoes for spring barley. This sequence of cultivations would be used had the potatoes been harvested in the autumn, left over winter and cultivated in the spring. This delay

before cultivation results in the soil becoming more compact, thus requiring ploughing. The ploughing operation was in the same direction as that of the cereal cultivations.

The spring cereal sequence resulted less microgranule spread than for the winter wheat sequence. This was to be expected from the results of the individual mouldboard plough cultivation and the study by Marshall and Brain (1999). However, the cumulative movement of the microgranules by the terra-disc and plough may have moved a large number of microgranules beyond the S to N axis. This is also suggested by the higher percentage of microgranules moved laterally for the spring barley sequence as opposed to that of the winter wheat sequence. This could mean that microgranules are being moved as far or further with the addition of the plough but not picked up using the sampling method adopted.

5.3.3.5 Movement within a crop rotation

Although the individual cultivation work found that the majority of the microgranules were moved by less than 1 m, some cultivations moved the microgranules over 4 m. It is important to consider each cyst as an individual focus point within a field. A new cyst can contain several hundred viable eggs. PCN even at low populations can multiply over 70 times on a potato root (Trudgill, 2003). Therefore, it is important that the movement of cysts by cultivation is not given as a singular predictive distance but as a range. For example with the bed tiller a cyst can be moved up to 5 m along the axis of cultivation depending on how it comes into contact with the tines. Also with different cultivations there is a potential for lateral movement. High levels of variation were found in this study for the movement of the microgranules; this could have been reduced by increasing the number of replicates

From the results it has been found that the potential for the movement of cysts in a field by cultivation is great. The movement must also be considered in terms of a whole rotation.

For example a rotation may have potatoes followed by three consecutive cereals before the next potato crop. The cultivations involved in a potato crop were found to have the greatest potential for movement of cysts. This movement is mainly restricted to the beds, therefore along one axis. The subsequent cereal cultivations if carried out at right angles to the beds, spread the cysts along another axis. Although these cultivations caused less range of movement, in a rotation they would be implemented more. The result of the this would be the spread of cysts after aggregation around a potato plant, in all directions. The aggregation of the cysts on potato plant roots would be spread along the bed by the harvester and subsequent bed flattening. The cereal bed preparations would then move the cysts predominantly at right angles to the beds. This would result in the spread of cysts in most directions mainly along the axis of cultivation. In a study by Been and Schomaker (1990) foci were found within a field and their shape determined by intensive sampling; they found that the shape of the foci was long and narrow following the direction of cultivation. The results of this work would suggest that this would be the case if cultivations were all carried out along the same axis. In a similar study Boag, Filipe and Niesten (2000) suggested that the PCN foci were more circular in shape. The shape of these foci suggests that the field had been cultivated at right angles for potato crops to cereal any subsequent crops with the cysts being spread in all directions, resulting in a circular shape. From these studies and the results presented shape of the foci will be dependent on the intensity of the cultivations carried out in a field and their direction.

Once PCN has been introduced into a field it will be moved by any subsequent cultivations; the range of the spread will be dependent on the cultivation machinery being used. The PCN will then be able to infest and reproduce on the next potato crop or volunteers, if in range of the potato roots. Any subsequent cultivations will spread the new cysts further into the fields. The potential for field wide spread of the cysts within several rotations is high. The aggregations during a potato crop and subsequent dilutions by

cultivation mean that the potential exists for the PCN cysts to be spread widely within a field before being detected. This agrees with Trudgill, Elliott, Evans and Phillips (2003) who stated that PCN may be present in a field for 30 years before it reaches a detectable level from canopy damage. If this is the case then given the range of spread found in this study by the cultivation operations upon detection of PCN the field should be managed as widely infested.

The use of different tillage systems may reduce the spread of PCN within a field and enable quicker detection of the problem. However, if potatoes are to grown, using a bed system, the cultivations carried out to implement this system are those that cause the greatest range of cyst movement. The cereal cultivations investigated caused a wide range of movement. Although cultivations to reduce the spread of cysts in a field could be implemented (such as direct drilling); PCN infestations first need to be detected. Also with a rotation involving beds some intensive cultivation is needed to flatten the bed to produce a suitable seedbed for other crops. A further consideration is that if PCN is present it may be of more value to spread out the PCN cysts in order to reduce its effects on individual potato plants and subsequently the crop yield.

From this work it would be possible to create a predictive model for the movement of cysts within a field. Although the resulting information would show a potential range of movement rather than give an absolute location, this could be of use for patch applications of nematicides, over several crop rotations. However, the detection of PCN in a field would need to be achieved before it was detectable in the canopy. After this point it is likely that the spread of PCN in the field would be extensive. This may mean the need for intensive sampling on any field going to have potatoes grown once PCN has been detected in any fields on a farm.

Chapter 6

General discussion

Potato cyst nematodes, unlike other species of nematodes, can persist for many years in the soil without a host crop. An understanding of PCN population behaviour, both spatially and temporally is, therefore, important for the development of the integrated management of PCN in commercial farming systems. The natural decline of PCN in the absence of a host crop and movement of PCN by cultivation operations will be discussed in turn. Also the interactions between these two areas of research will be discussed. In addition, recommendations for the practical implications of the results will be suggested.

Natural decline of PCN

The success of crop rotation as a control method is dependent on the natural decline of PCN in the absence of a host crop. During the past fifty years a number of studies have been undertaken to quantify the decline of PCN and thus be able to predict the length of rotation required for infested land (e.g. Cooper, 1953; Cole and Howard, 1962a; den Ouden, 1974; Wharton, 1985; Whitehead, 1995). The decline rates found in these studies have ranged from between 10 and 60 % per annum. The implications of this range of decline, in relation to effective management incorporating rotation control, are great. Direct comparison between these studies is problematic due to differences in species present and experimental methodologies.

On the basis of existing literature, it was clear that a standardised methodology was required to investigate whether the apparent range of declines is present in different PCN populations. In this project, both field and plunge pit experiments were employed to produce a standardised method for monitoring the decline of PCN in the absence of a host crop. Subsequent analysis of the data was undertaken to determine the factors responsible for variation in the decline rate of PCN populations.

Four years of sampling semi-permanent field test stations (section 2.3) revealed problems with in-field monitoring of PCN decline rates. In 2000, increased sampling intensity within the test stations found high levels of variation in PCN population densities. The results also

showed large levels temporal variation within the test station PCN population densities; these included increases in PCN populations in the absence of a host. This is not possible due to PCN requiring a host plant to complete its life-cycle. The sampling of field soil in plunge pits did not show these increases. This suggested that the PCN population densities within the test station were being affected by factors other than their natural decline. Subsequent analysis of cyst number found that they also increased in the test stations which had shown population density increases. The number of cysts although, likely to decline slower than that of the PCN eggs, should not increase. The factor responsible for these results was likely to be the spatial changes in the PCN population densities in and around the test stations. Field cultivation operations were probably responsible for this movement of PCN population densities within the field. This was confirmed by the results of the Plunge pit experiment where soil was removed from infested fields and subsequently sampled. These PCN population densities did not exhibit increases. Experiments reported in Chapter 5 confirm that PCN cysts in soil are moved by cultivation operations. Numerous cultivations were carried on the fields between sampling dates.

The results of this experiment suggest that the use of point samples or sample areas in fields will provide estimates of population densities for a location but that these are likely to alter temporally following cultivation operations. Although this experimental method was not successful for determining field declines of PCN, it does raise questions regarding past studies. Decline studies carried out in fields over a number of cropping years will be affected by the cultivations involved. Turner (1996) found that decline rates for infested fields in Northern Ireland over 13 years were erratic, she put this down to variable cyst ages and large standard errors at low population densities. It was found that regular cultivation of infested land appeared to accelerate decline. The acceleration of decline under regular cultivation could have been due to the dissipation and mixing of cysts by

cultivation. This would result in variable cyst ages between the sampling dates and also the observed large standard errors.

Cooper (1953) found that PCN in fallow fields declined at a lower rate than in fields with a cereal grown, and suggested that crop type was having an effect on the decline rate. Whitehead (1995) carried out this same comparison using microplots and found no significant difference in the decline rates for the two treatments. He suggested that the reason for the differences in decline observed by Cooper (1953) were due to ploughing speeding up the decline of PCN in the cereal fields, due to this not being a factor in his microplot design. The reason for the observed decline rate difference is more likely to have been due to the horizontal movement of cysts by cultivation. There have been no studies carried out investigating the direct effect of cultivation on the physical damage or decline of PCN. In India it has been found that multiple summer ploughings reduces population densities of the cereal cyst nematodes (*Heterodera avenae*) (Mathur *et al.*, 1991). However, they attribute the decline to the cysts being brought to the surface and exposed to solar heat. This is unlikely to be a major contributing factor when single ploughing in the UK. In addition to this the increased yields of cereal following this summer ploughing may be due to the dispersal of cysts by multiple ploughing as well as their destruction by solar heat i.e. if the cysts are widely dispersed then invasion of individual plants may not reach a damage threshold.

The declines studies conducted using Plunge pits with, PCN infested, soil removed from the field and showed decline rates ranging from 11 to 69% per annum. The variation in decline rates between the populations could not be accounted for by differences between *G. pallida* and *G. rostochiensis* populations or completely by differences in soil type. Although, all PCN populations were collected at the same time after a potato crop to ensure populations of the same age this may not be the case. The removal of soil from the field removed the direct spatial effects of cultivation operations on the population

dynamics. However, cultivation operations carried out in the fields before the soil was collected could be the reason for the variations in the decline rates. The direction and extent of movement of cysts by a cultivation operation vary depending on the machinery used and its purpose (Chapter 5). This will result in variations in the amount of mixing of cysts within the soil. PCN require host plant roots to complete their life-cycle however, the formation of beds results in some cysts hatching near potato roots and others away from the roots in the furrow, or not being stimulated to hatch. Following a potato crop these areas are mixed resulting in PCN population densities of varying aged cysts. The location of the fields sampled and the ratio of cyst ages could have resulted in the high variations of decline rates. However, knowing the decline rates of the mixed aged populations is important for determining the effects of rotation length on a field population.

A major limitation of the plunge pit experiment was one of duration (eighteen months); this constraint meant that only one annual decline rate was recorded for the PCN populations. This meant it was not possible to compare the results with previous studies (such as Huijsman, 1957; den Ouden, 1960 and Stone *et al.* 1973) where the annual decline rates were found to be greater in the first year after a potato crop. Some evidence was found for this in the plunge pit populations when comparing the declines during the first and second growing seasons. Continuing to sample the plunge pits on an annual basis would provide comparable results with the previous studies.

From Chapter 2 it is evident that the removal of soil is necessary for estimating PCN decline rates. However, the observed variation between the PCN populations, suggest that this it is necessary to determine PCN decline rates for infested fields growing potatoes on an individual basis. Within-field sampling should also be implemented prior to a potato crop. The use of both techniques would provide information on the population within the field and additionally the decline rate that can be expected from that population. The setting up of a plunge pit post potato crop would allow the collection of several years

decline data before the next potato crop in the rotation. If several plunge pits were setup with soil from different locations the potential for variation in age of cysts in the pits relative to the field populations could be reduced. The collected soil should be contained near the actual field to take into account any *in situ* environmental factors.

Cyst movement by cultivation operations

Although cultivation operations are widely accepted as a major source of PCN spread within a field (e.g. Turner and Evans, 2000; Been and Schomaker, 1996; Boag, Filipe and Niesten, 2000) no previous studies have been conducted to quantify it. Initially it had been hoped to determine PCN movement by cultivation operations, using changes in PCN population densities during cultivation operations. However, the experiment conducted in Chapter 4 on the movement of cysts suggested that this would be difficult. This initial experiment was able to show that movement was occurring but not quantify the distances or number of cysts moved. The experiment investigated the lateral movement of PCN within a bed, by the actions of a bed-tiller and harvester, this was along one axis. To evaluate the movement of cysts using a range of cultivations requires monitoring potential movements along multiple axes. The net movement of PCN cysts from other locations could also result in the potential for obscuring any movement taking place. The variability in PCN populations densities over short distances, as found within the test stations in Experiment 1 (chapter 2), could also result in problems with replication. This meant that the use a cyst-substitute was necessary, which required validation before it could be used. Following validation, microgranules were selected for the subsequent movement studies. The experiments showed that all cultivation operations had the potential to move cysts. This movement was not restricted to the direction of cultivation; lateral and backward movements were found. The cultivations producing the greater lateral movement were those involving tractor powered machinery. The cultivation operations during a potato crop

caused the most movement within the soil. However, these were mainly along the axis of cultivation due to the movements being restricted to within the beds. The cultivations investigated which were not employed during a potato crop resulted in less movement. However, within a potato crop rotation these operations are likely to be employed more often. Therefore, these operations are potentially more important for spreading PCN within a field. Growers usually cultivate and plant potatoes in a north/south direction, while cultivating cereals in an east/west direction. This means that the PCN will be spread out across the field and not restricted to the lines where the potato beds were. Boag, Filipe and Niesten (2000) suggested this as the reason for finding that PCN foci within a field were relatively circular in shape.

From the data obtained in Chapter 5 it had been hoped to construct a predictive model for the movement of cysts within a field. However, this was not possible due to time constraints. The resulting model could have been used to predict the spread of PCN in a field under different crop rotations. The cultivation machinery utilised could also be factored into the model to estimate the resulting effects of using specified cultivation machinery, within a given rotation. Additionally, the model could be made spatially dynamic for a field if initially intensively sample for the detection of PCN foci. The model could be used as useful educational tool for growers showing the potential for PCN spread within a field and therefore, the need for early detection.

Management of PCN in the UK is heavily dependent on the use of nematicides. However these are a costly input with granular and fumigant nematicide costing £ 360 and £ 550 ha^{-1} respectively (Anon., 1998). The patchy distributions of PCN and increased accuracy in GPS mapping technology have resulted in the investigation into site-specific application of nematicides (Haydock and Evans, 1995). The accuracy of the PCN maps is dependent on the intensity of sampling undertaken on a site. The costs for producing the PCN maps increases with sampling intensity. This would lead to potential limitations in the practical

application of this method (Evans *et al.*, 2002). The potential exists to use the PCN field maps for a site within the predictive model for cyst movement by cultivation. This could make the PCN maps spatially and temporally dynamic and be used to predict patch treatment with nematicides. This would reduce the need for frequent sampling and could therefore, reduce the costs of implementing patch applications of nematicides. However, the accuracy of predictive PCN mapping will be dependent on the information provided in the initial PCN field map. The current method for sampling fields for mapping PCN population is by point sampling a field at intervals of 20 m on a square grid. The use of field test stations in Chapter 2 found variations between point sampling and sequential bulk sampling within a small area (2 m^2). The subsequent sampling intensification experiment confirmed that the variations within the area were responsible for the differences in the estimates for PCN population densities within the area. This suggests that the collection of point samples may not provide reliable information on the PCN population for a given area. Evans *et al.* (2003) also found evidence of spatial independence in PCN counts at a range of 10 to 20 m. The collection of bulked samples from within an area may be required to produce more accurate information on the PCN population densities to construct the initial PCN maps. A similar level of sampling intensity, as for the point sampling could be employed, to produce accurate maps.

6.2 Conclusions

Natural PCN decline studies

- The variations in decline rates observed in previous studies do occur between PCN populations from different fields.
- The use of field sampling to predict PCN decline is not reliable if cultivations are carried out between sampling dates.
- Neither *Globodera* spp. present nor soil type were found to be responsible for the variations in declines rates.
- The placement of field soil in plunge pits next to a field will provide the grower with site specific information of decline to factor into his integrated management strategy. Collection of the soil from numerous points of the field should increase the potential for the plunge pits to contain an age ratio of cysts comparable to that of the field.

PCN movement by cultivation operations.

- PCN is spread in fields by cultivation operations.
- The cultivation used and its purpose determine the direction and extent of cyst movement.
- The spread of PCN, and subsequent dilution, by cultivation could increase the duration between initial infestation and detection.
- On detection PCN is likely to be widespread within a field.

6.3 Future work

- Use the mixed populations to investigate whether there is a significant difference in the declines of the *Globodera* spp. The ratio between the two species could be monitored to determine if one species is declining at a higher rate. This has the advantage that the soil type and other soil factors will be the same for both species. The monitoring could be done by soil sampling and then carrying out PCR quantification.
- The plunge pit populations could be used as basis for an investigation into whether the age ratio of cysts is an important factor in determining a populations decline rate. Cysts extracted from the plunge pits could be added to pots with susceptible cultivars to allow the PCN to multiply. If the cysts used were placed in a bag in the pots it would allow juveniles out but not the cysts. The new populations decline could then be compared without the factor of different cyst age ratios.
- Using the data collected from the cultivation studies, a computer simulation model could be developed to show the potential spread of PCN within a field. This could be interactive where the user can input the rotation and cultivation machinery to be employed.
- In addition to this the cultivation work should be carried out on different soil types to investigate the potential effects they have on the spread of PCN in a field.

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